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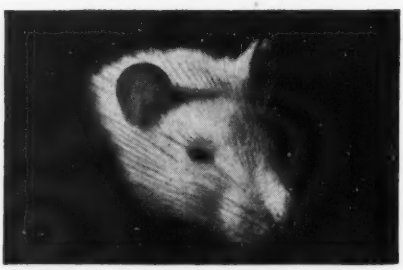
WHY



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[1] Harrington, C. R.: "The Thyroid Gland," Oxford, 1933, p. 141.
[2] Meyer, A. E., and Wertz, A.: Endocrinology 24: 806, 1939.

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INSULIN INHIBITION BY SERUM OF INSULIN RESISTANT PATIENT

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INSULIN resistance is, fortunately, a rarity among diabetics. However, in diabetic coma it may be quite common depending on one's definition. In the literature fewer than 50 cases of true insulin resistance are recorded (2, 3, 9–12, 14, 16–18).

We have one more case to add to this small but significant group, and this case was distinguished from many others (2, 11, 12, 17) because an insulin inhibiting factor was detected in the serum of the patient at the height of insulin resistance.

CASE HISTORY

The patient was a 79 year old female, who for six years had mild diabetes and hypertension. Glycosuria was controlled by diet alone, until she developed gangrene of the right foot incident to an infected laceration. Polyuria and polydipsia developed, and she excreted as much as from six to seven per cent glucose. At the time of admission to the Montefiore Hospital, October 20, 1944, she had a four plus acetonuria; the blood sugar was over 400 mg. per cent; the CO_2 content of the serum was 54 volumes per cent; and serum chlorides were 343 mg. per cent.

A total of 85 units of zinc crystalline insulin soon rendered her urine free from sugar and acetone. She was then maintained on 10–20 units of insulin per day with a diet of 150 grams of carbohydrate, 80 grams of protein and 100 grams of fat.

On the sixth hospital day a mid-calf amputation was performed by Dr. Daniel Rock, and following this she required small amounts of insulin until the tenth postoperative day, when acetone and from two to three per cent glucose appeared in the urine. Gangrene of the stump developed. The insulin was gradually increased to 100 units per day.

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but despite this, acetone again appeared in the urine and on the 26th postoperative day, signs of coma were evident. At this time her blood sugar was 480 mg. per cent; CO_2 content, 29 volumes per cent; and serum chlorides, 350 mg. per cent. A total of 1200 units of insulin was required over a 24 hour period to render her urine acetone free and to raise the CO_2 content to 46 volumes per cent. At this time a mid-thigh amputation was performed by Dr. Joseph Alper.

Following the operation acetone again appeared in the urine, and from 200 to 1100 units of insulin were required daily. Hourly tests of the urine for glucose and acetone were performed and the insulin dosage regulated accordingly. During this period of insulin resistance the patient's food intake was inadequate. We have no exact measure-

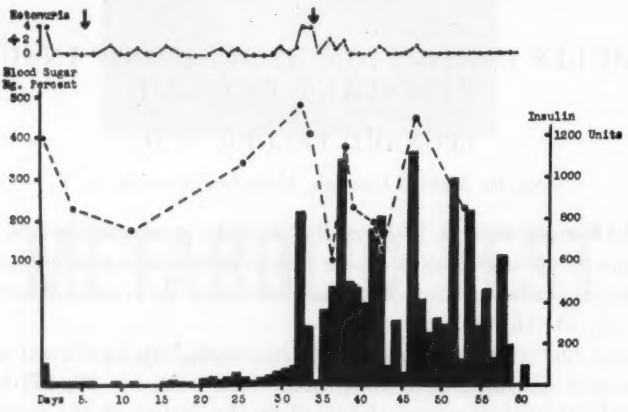

FIG. 1. Daily insulin requirement, fasting blood sugars (large dots) and daily ketonuria (small dots).

ments of this. We have very few quantitative urinalyses for glucose; those which we have range between 25 and 50 grams excreted per 24 hours (Figure 1).

An **insulin tolerance test** was performed on the 42*nd* hospital day, 50 units of crystalline zinc insulin being injected intravenously. The blood sugar fell from a fasting level of 138 mg. per cent to 127 mg. per cent at the end of one hour. In three and one half hours the blood sugar had risen to 150 mg. per cent (Figure 2).

Gangrene of the left heel and a decubitus ulcer were noted about three days after the second operation. The decubitus ulcer later suppurated. There were no other intercurrent infections and the stump of the second amputation healed well. The patient died on the 61st hospital day.

Other laboratory data: Blood pressure 170/70 mm. of Hg.; hemoglobin, 11 gm.; erythrocytes, 4 million; leucocytes, 20,250; polymorphonuclear cells, 68 per cent; band forms, 16 per cent; lymphocytes, 16 per cent. The specific gravity of the urine was 1.012–1.028; there was a faint trace of albumin, and there were rare red cells, from two to ten white cells, and occasional hyaline and granular casts; the blood urea nitrogen was 25 mg. per cent; urea clearance, 72 per cent; cephalin cholesterol flocculation, negative; plasma cholesterol, 173 mg. per cent; plasma albumin, 3.3 gm. per cent; globulin,

2.3 gm. per cent; Wassermann, negative. The electrocardiogram showed left axis deviation with intraventricular conduction disturbance.

Roentgenograms of the chest showed left ventricular enlargement, dilatation and calcification of the aortic arch, and prominent pulmonary arteries. The skull showed generalized decalcification.

Post mortem examination, performed by Dr. David Unterman and Dr. Samuel H. Rosen, revealed severe generalized arteriosclerosis and arteriolosclerosis; slight hypertrophy of the heart with patchy myocardial fibrosis of the left ventricle; small organizing mural thrombi in left and right ventricles; arteriosclerosis of the pulmonary arteries with multiple organized thrombi in the smaller branches, a recent thrombus in branch to the right lower lobe, and a small recent infarct in the right lower lobe. The lungs were congested and edematous, and there was a left pleural effusion. The liver weighed 1410 gm. It was soft, pale brown, and the lobules were swollen. Microscopically it showed

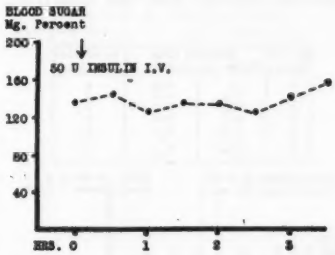

FIG. 2. Insulin tolerance curve after injection of 50 U of crystalline zinc insulin intravenously.

central congestion, occasionally with central necrosis of the lobules. The liver cells were swollen, pale and coarsely granular in varying degree and frequently had vacuolated nuclei. With Best's carmine stain numerous smaller and larger areas throughout the section showed liver cells containing scattered to closely packed bright-red stained granules of glycogen. Other liver cells contained no stainable glycogen or only fine scattered granules. Many nuclei, not necessarily the markedly swollen vacuolated ones, also contained the bright red staining glycogen granules. Focal fatty metamorphosis was shown by the Sudan IV stain to be moderately extensive with no special localization in the lobules. There was slight increase in size of the portal spaces with rather marked infiltration of small round cells. With respect to the glycogen content of the liver, the pathologist made the following observation: "In view of the fact that the tissue was taken six hours or more postmortem, and that it was not specially fixed for glycogen staining (fixed in 10 per cent formalin and in Orth's solution and stored in 80 per cent alcohol for about six months) the considerable amount of glycogen still demonstrable under these unfavorable circumstances probably indicates that there was much more glycogen storage in the liver in life."

The **pancreas** showed nothing abnormal grossly. Microscopically the two sections taken showed hyaline degeneration of the islets of varying degree, usually marked, and involving most of the islets. There was marked hyaline sclerosis of the arterioles. A few aberrant nests of pancreatic acinar tissue were present in the wall of the duodenum.

The **kidneys** weighed 135 gm. (right) and 110 gm. (left) and were granular. Microscopically they showed many scars, moderate to marked hyaline sclerosis of arterioles and marked sclerosis of arteries. Most glomeruli showed thickening of capillary walls of varying degree. There were no definite "hyaline-ball" lesions of intercapillary glomerulosclerosis.

The **spleen** contained two old infarcts and the splenic artery was markedly arteriosclerotic.

The **adrenals** weighed 5.5 gm. each and were mostly lipoid-poor, though focally lipoid-rich. There was hyaline sclerosis of pericapsular and capsular arterides.

The ovaries were atrophic and contained many corpora albicantia. The neck organs and brain could not be examined.

RESULT OF ANIMAL EXPERIMENTS

We attempted to demonstrate in mice an inhibition of insulin by the patient's serum. All insulin, except for the morning dose of 80 units, was

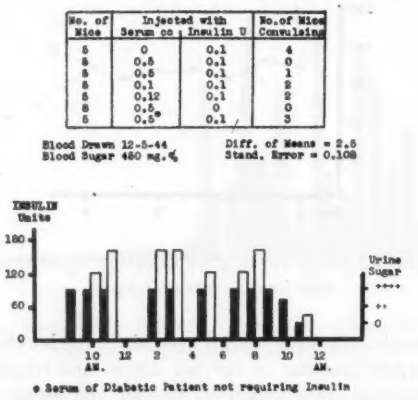

FIG. 3. Results of animal experiments exhibiting insulin inhibition by the patient's serum. The hourly insulin requirement (white areas) and glycosuria (shaded areas) for December 5th, 1944, are depicted below.

withheld from the patient on December 4, 1944, and 24 hours later blood was drawn, allowed to clot, centrifuged and the serum separated. White albino mice, weighing from 20–30 gm. each, were fasted for 18 hours, kept at a temperature of 35–37 degrees centigrade, and injected intraperitoneally with 0.5 cc. of serum, plus 0.1 unit of insulin (zinc crystalline) without incubation of the mixture. This was the method used by Lowell (10). However he used 0.03 unit of insulin and Schrier (14) used 0.02 unit of insulin in his experiments. We found that hypoglycemic convulsions could not be produced in our mice with less than 0.1 unit of insulin. This experiment was performed on three occasions over a period

of 15 days. Serum from another diabetic patient, who was not receiving insulin, was used as a control. In the control series there were hypoglycemic convulsions in seven out of ten mice. Using the patient's serum there was a convulsion in only one out of ten mice. When the serum was diluted 1:4 and 1:5 there were convulsions in four out of ten mice. These figures are statistically significant, but the series is small.

Figure 3 shows the results of these experiments and the patient's insulin requirement and urinalysis of that day.

No. of Mice	Injected with Serum cc	Insulin U	No. of Mice Convulsing
5	0	0.1	4
5	0.5	0.1	4
5	0.12	0.1	3
5	0.12	0.1	2
4	0.5	0.1	1
5	0.5	0.1	2
6	0.5a	0	0
5	0.5a	0.1	4

Blood Drawn 12-11-44
Blood Sugar 240 mg. %

Diff. of Means = 1.35
Stand. Error = 0.117

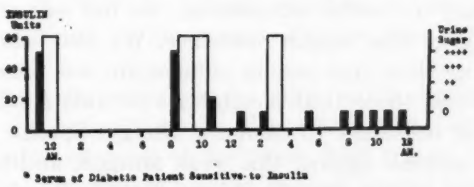

FIG. 4. Results of animal experiments exhibiting mild insulin antagonism by the patient's serum. The insulin dosage (white areas) and glycosuria (shaded areas) for December 11th, 1944, are depicted below.

These tests were repeated on December 11, 1944. The patient, however, was given 1000 units of insulin the day prior to the experiment. The serum of this day inhibited hypoglycemic convulsions in 7 out of 14 mice when 0.5 cc. of serum and 0.1 unit of insulin were used. With dilutions 1:4 of serum and 0.1 unit of insulin there were convulsions in five out of ten mice. The control group showed convulsions in eight out of ten mice. These experiments were performed over a period of 19 days after obtaining the serum (Figure 4). Similar experiments on post-mortem serum performed 30 days after death showed no insulin inhibitory effect. Attempts to effect a positive passive transfer or to find precipitins in the post-mortem serum were negative; they were carried out 90 days later.

It is of interest to note the correlation between insulin requirements on the two days that the serum was drawn and the experimental results. We may conclude from these experiments that on December 5, 1944, the

patient's serum inhibited the hypoglycemic effect of insulin and that on December 11, 1944, there was much less inhibitory action.

DISCUSSION

The term insulin resistance indicates that the patient must require more than 200 units of insulin per day, for more than 48 hours. This definition, proposed by Martin, Martin, Leyster and Strouse (11) in their review of the subject, is based on the calculated requirement for a totally depancreatized man as deduced by Root (13). However, Goldner and Clark (4) and others (1) have reported cases following total pancreatectomy that received surprisingly little insulin, about 50 units a day. Lerman (9) in a study of insulin allergy believes that from 50 to 60 units of insulin are required by a totally diabetic human. He bases his assumption on the clinical fact that insulin allergic patients when desensitized finally require this amount of insulin. He suggests, by analogy, that a person immunized to insulin loses completely his capacity to produce insulin and, therefore, becomes permanently and totally diabetic. In spite of the marked variations in insulin requirement, we feel safe in assuming that our case was one of true insulin resistance. We also believe that our experiments on mice show that insulin antagonism was present.

There are several theoretical possibilities for this condition. The most common cause of resistance to insulin is allergy. Specific antibodies may be produced in animals against this weak antigen, and have been found in man (6, 9, 10). In the absence of any clinical allergic manifestations, the failure to find precipitins in the patient's serum, and our inability to effect a positive passive transfer, it does not seem likely that allergy was the factor responsible for insulin antagonism in this case. The possibility of inadequate absorption was excluded by administering the insulin intravenously and intramuscularly. Impotency of the insulin could not play a part as the same insulin was used on other diabetic patients and on the mice and was found to be effective. While we did not rule out the effect of an excess of some enzyme which could increase glycogenolysis (15), we did show that the ineffectiveness of insulin was not due to the absence of a substance needed to potentiate insulin activity. If there had been a lack of an insulin kinase, as Himsworth (7) postulated, then the normal animal should have reacted to the large dose of insulin given.

As there was no direct evidence of pituitary or adrenal involvement, we need not consider these glands as contributory elements to the inhibition of insulin in this case. However, the role of infection in insulin resistance cannot be overlooked. Greene and Keohen (5) reviewed this subject. Cirrhosis or other hepatic dysfunction may cause either hypo- or hyperinsulin sensitivity (8). If glycogenolysis is markedly impaired, it is

feasible that large amounts of insulin may be required to prevent acetonuria due to the loss of glucose and thus increased fat metabolism. But this would not explain the presence of an anti-insulin substance in the blood.

Therefore, we may assume that the lack of response was due to neutralization of the insulin either by destruction, combination or direct antagonism. From our experiments on mice, we know that the responsible agent was present in the patient's serum.

SUMMARY AND CONCLUSIONS

1. A case of insulin resistance is reported. The patient developed her insulin antagonism after amputation of a gangrenous foot. Gangrene of the stump necessitated another amputation and a large suppurating decubitus ulcer further complicated the case. She required from 20 to 1120 units of insulin per 24 hours, with average daily doses of from 400 to 500 units. An insulin tolerance test with 50 units of crystalline zinc insulin given intravenously did not lower her blood sugar significantly.

2. The patient exhibited no allergic reactions to the insulin and precipitins were not found in her serum. Passive transfer was attempted without success.

3. Experiments were performed on mice which demonstrated insulin antagonism by the patient's serun. The anti-insulin factor was demonstrable 19 days after drawing of the blood, with 0.5 cc. serum inhibiting 0.1 unit of insulin. The patient's serum showed greater insulin antagonism when insulin was withheld for 24 hours than when insulin was given freely.

4. The theoretical possibilities for the cause of insulin resistance in this case were discussed.

5. It was shown that insulin resistance could be overcome by giving sufficient quantities of insulin.

I wish to thank Dr. Louis Leiter, Chief of the Medical Division, Montefiore Hospital, for his helpful suggestions and encouragement.

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HORMONE METABOLITES IN BLOOD AND URINE OF DIABETIC PREGNANT PATIENTS WITH AND WITHOUT TOXEMIA

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IN 1934, Smith and Smith (20) reported that hormone metabolite concentrations in the urine and blood of toxemic patients differed from those found in normal pregnancy. The differences observed by these workers consisted in an increase in serum and urine levels of chorionic gonadotrophin and a decrease in serum and urine levels of estrogen. Browne, Henry and Venning (6), in 1938, reported a lowered excretion of pregnanediol associated with toxemic pregnancy. Since then these early reports have stimulated a number of investigations of the hormone metabolite levels in toxemic pregnancy. Among the contributors to this field are Bachman and his co-workers (1, 2, 3), Taylor and Scadron (28), Watts and Adair (32), and White and her co-workers (34–36). Normal levels have also been studied extensively by these workers, by Cope (9), and by Jones (15) and her co-workers.

A summary of the results of investigations by various workers may be seen in Table 1, which was compiled in the following manner: in the column headed "Number of Toxemics" only those patients were considered for whom individual data were reported. The values of the various hormone levels reported for these patients were compared with the range of normal values given by the same investigator, either in the same paper or in other papers, and called low or high if they were outside the range of normal values in either direction. Values before hormone therapy was instituted were used as reference in treated cases. The percentages are based on the number of toxemic patients, without regard as to whether or not they were also diabetic, as no difference was observed between diabetic and non-diabetic toxemics. This was also true for diabetic and non-diabetic patients who had normal pregnancies.

Examination of Table 1 leads to the conclusion that toxemia of pregnancy is often associated with an abnormal hormone metabolite pattern. Bachman (2), Browne and his co-workers (6), and Smith and Smith (21–28) found a lowered excretion of pregnanediol in nearly 100 percent of the toxemic cases they studied, White and Hunt (34) found lowered preg-

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nanediol in 20 percent of their toxemic cases. Low urine estrogen was found by Browne (6, 7), Lu (16), Siegler (19), the Smiths (21–28), and Watts and Adair (32), in percentages ranging from 48 to 100 percent of the cases studied, but Taylor and Scadron (29) found low urine estrogens in only 28 percent of their cases. Low serum estrogen was found in from 17 to 33 percent of the toxemic patients. Smith and Smith (21–28), Siegler (19), and White and her co-workers (34, 35) found high serum and urine gonadotrophin in from 61.5 to 100 percent of toxemic cases, but Taylor and Scadron (29) and Watts and Adair (32) found the occurrence of high serum

TABLE 1. SUMMARY OF LITERATURE CONCERNING CONCENTRATIONS OF PREGNANEDIOL, ESTROGENS, AND CHORIONIC GONADOTROPHIN IN TOXEMIC PATIENTS

References	Number of normals	Number of toxemics	Fetal survival (toxemic patients) per cent	Changes in toxemic patients								
				Pregnanediol	Estrogen				Chorionic gonadotrophin			
					Urine		Serum		Urine		Serum	
				low values per cent	per cent high	per cent low	per cent low		per cent high	per cent low	per cent high	per cent low
Bachmann, Leekley & Hirschmann (2)	6	3	100	100	—	—	—		—	—	—	—
Browne, Henry & Venning (6)	8	21	—	"most"	—	"most" (exceptions)	—		"most normal tending to low"		—	—
Lu (16)	87	24	—	—	4	62	—		—	—	—	—
Siegler (17)	5	1	—	—	0	100	—		100	0	—	—
Smith & Smith (21–27)	70	100 13[1]	36 untreated 83 treated	ave. lower ave. lower	10	67	17[2]		85[3]	0	84.0 61.5	0
Smith, Smith & Hurwits (28)	16	8 4[1]	50 50	100 100	0	100	—		—	—	75.0	0
Taylor & Scadron (29)	17	18	72	—	0	28	33		11	0	0	0
Watts & Adair (32)	13	25	—	—	0	48	24		33	0	28.0	0
White, Titus, Joslin & Hunt (36)	6	25 3[1]	75	—	—	—	—		—	—	64.0 100.0	8 0
White & Hunt (34)	43	14 11[1]	65	20	—	—	—		—	—	74.0	0

[1] Premature delivery.
[2] 17 per cent of 18 cases studied.
[3] 85 per cent of 48 cases studied.

gonadotrophin to be 0 to 28 percent and of high urine gonadotrophin to be 11 to 33 percent.

The present report consists of a study of the serum and urine chorionic gonadotrophin and of estrogen and pregnanediol excretion in five pregnant diabetic women throughout the latter part of pregnancy. Of these five women, two developed toxemic symptoms but were delivered of normal babies, while three had asymptomatic pregnancies with respect to toxemia, but one of the latter group was delivered of a macerated fetus, and the infant of another died within a few hours of birth.

EXPERIMENTAL SUBJECTS

Case 1: Mrs. M., aged 25 years, had severe diabetes of three years' duration, and was maintained on 50 units of insulin per day. During a pre-

vious pregnancy the patient developed toxemia, and was delivered at term of an 11 pound stillborn infant.

Case 2: Mrs. DaL., aged 20 years, had severe diabetes of seven years' duration and was maintained on 110 units of insulin per day. This was her first pregnancy.

Case 3: Mrs. K., aged 37 years, had moderate diabetes for five years, and was maintained on 25 units of insulin per day. A previous pregnancy ended in stillbirth at eight months.

Case 4: Mrs. L., aged 19 years, had severe diabetes for four years, and was maintained on from 60 to 145 units of insulin per day. A previous pregnancy ended in stillbirth at eight months.

Case 5: Mrs. T., aged 26 years, had severe diabetes for thirteen years, and was maintained on 70 units of insulin per day. A previous pregnancy ended in miscarriage at six months. Severe pyelitis eight years previously left no apparent residue.

METHODS

Urine and blood samples were collected at intervals of two weeks until the end of the seventh month of pregnancy, and thereafter at weekly intervals. When clinical signs of impending toxemia were observed, such as hypertension, excessive gain in weight, or subjective symptoms, the patients were hospitalized and daily specimens were obtained. The urine, usually 24 hour samples, was preserved with toluene. Twenty-five cc. portions of the urine and 15 cc. of cell-free serum were stored at $-8°$ C. until gonadotrophin assays could be run. Urine protein was determined immediately by the method of Shevsky and Stafford (18), sugar by the method of Benedict (4), and creatinine by the method of Folin (11), this latter in order to afford a means of checking the accuracy of the twenty-four hour collection (20). The urine was extracted for pregnanediol and estrogen within 24 hours of collection.

The pregnanediol concentration of the urine was determined by the gravimetric method of Venning (30). The urinary estrogens were extracted and separated into fractions containing estriol and estrone-estradiol by the method of Bachman and Pettit (3). These fractions were assayed by the colorimetric method of Venning, Evelyn, Harkness and Browne (31). The estrone-estradiol fraction was assayed in terms of estrone, while the estriol fraction was assayed in terms of estriol. All values for estrogens are corrected values according to the method of Gibson and Evelyn (12). Readings were made at 420 and 522 millimicra respectively. Samples of urine collected on the patients after termination of pregnancy served as "estrone-free" urines for use in the correction formula.

For the preparation of solutions suitable for assay of chorionic gonado-

trophin in serum or urine, 10 cc. of either fluid was treated with 100 cc. of 95 percent ethanol in a 250 cc. centrifuge bottle and allowed to stand overnight at 5° C. The supernatant was discarded and the precipitate washed with 100 cc. of ethyl ether. After most of the ether had been removed by passing air over the precipitate, about 5–10 cc. of normal saline were added to the tube. The precipitate was thoroughly wetted with the saline, and then the volume made up to exactly 25 cc. with additional saline. The remaining ether was removed by passing a stream of air over the solution

TABLE 2. UTERINE RESPONSE TO INTERNATIONAL STANDARD CHORIONIC GONADOTROPHIN AND TO SERUM STANDARD CHORIONIC GONADOTROPHIN

Total dose international standard I.U.	Total dose serum standard A.U.[1]	Number of rats	Mean uterine ratio (uterine wt. in mg.) / (body wt. in gm.)
0	0	49	0.60 ±0.03[2]
0.25	0	19	0.56 ±0.08
0.37	0	20	0.83 ±0.09
0.50	0	20	1.49 ±0.10
0.75	0	20	2.05 ±0.12
1.00	0	21	2.62 ±0.12
1.50	0	20	2.35 ±0.06
0	0.75	20	0.71 ±0.02
0	1.50	20	0.83 ±0.07
0	2.00	20	1.04 ±0.09
0	2.50	20	1.17 ±0.12
0	3.00	20	2.44 ±0.07
0	4.00	10	2.36 ±0.23
0	5.00	10	2.13 ±0.07

[1] Arbitrary Serum Gonadotrophin Unit equal to activity of 0.004 cc. of standard serum.

[2] Standard Error of Mean.

until no ether was detected. The tubes were centrifuged to remove undissolved protein, and the supernatant saline solution containing the chorionic gonadotrophin was stored at −8° C. Dilutions for bio-assay were made from these stock solutions.

For the bio-assay of the chorionic gonadotrophins immature female rats of the Sprague-Dawley strain, from 21 to 24 days of age, and weighing from 35 to 50 gm., were employed. The endpoint consisted of the weight response of the uterus. The total dose per animal was dissolved in 6 cc. of saline. Two doses daily of 1 cc. each were administered subcutaneously,

at least four hours apart, for three consecutive days. On the fifth day (approximately 96 hours after the first injection) body weight and uterine weight were determined.

A dose-response curve for the international standard of chorionic gonadotrophin was established using 49 rats which received only the solvent and 120 rats which received total doses of international standard ranging from 0.25 I.U. to 1.5 I.U. The responses are represented in Table 2, which shows increasing responses to increasing doses from 0.25 I.U. to

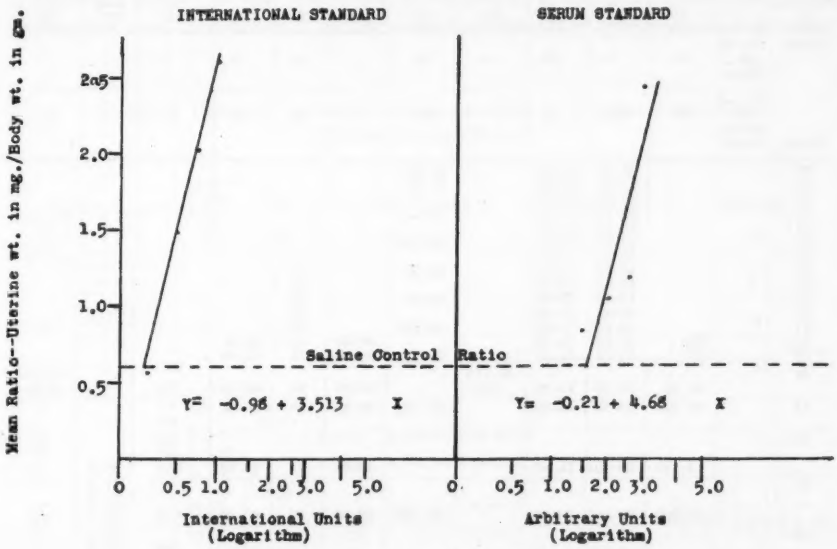

FIG. 1. Dose-response curves for chorionic gonadotrophin.

1.0 I.U. When the log dose-response functions are considered according to the method of Fisher (10) an equation of $Y = -0.98+3.513\ X$ was found (Figure 1).

A second dose-response curve was determined for a pregnancy serum. The amount of gonadotrophic activity present in 0.004 cc. of this serum was designated as one arbitrary unit (A.U.) of activity. A total of 120 animals were used at seven concentrations ranging from 0.75 A.U. to 5.0 A.U. Between 1.5 A.U. and 3.0 A.U., increasing doses of extract caused increments in uterine weight. On the basis of log 10 X dose versus response an equation of $Y = -0.21+4.68\ X$ was found (Figure 1).

An analysis of the slopes of the urine and serum curves by the method of Fisher (10) showed a significant difference in slopes ($P = 0.02-0.05$).

Therefore, a smaller error could be expected if the blood gonadotrophin values are expressed in terms of serum standard rather than the international standard. For this reason our data are reported in arbitrary units.

TABLE 3. URINARY EXCRETION OF PREGNANEDIOL IN NORMAL PREGNANCY
(Values in mg./24 hr.)

Duration of pregnancy	Reference:	Bachman (1)	Browne Henry & Venning (7)	Cantor (8)	Cope (9)	Jones (15)	Weil (33)	Smith & Smith (26)	White & Hunt (35)	Hain (13)	Wilson Randall & Osterberg (37)	Taylor & Scadron (29)
	No. of patients	6	8	10	—	18	—	14	9	4	1	—
Weeks	No. of observations	76	192	—	—	154	—	63	25	29	12	14
20			22–52	75–80		17–30						
21			22–52	75–80		17–28						
22			22–52	75–80								
23			22–52	75–80		19–55						
24			40–72	75–80								
25			40–72	75–80		20–120						
26			40–72	75–80								
27			40–72	75–80		28–80		50				
28			48–85	75–80				50				
29			48–85	75–80		40–90		50				
30			48–85	75–80				50				
31			48–85	75–80		25–100		50				
32		80–	55–95	75–80			60–80	75	60–80			
33		120–	55–95	75–80		60–130	60–80	75	60–80			
34					30–110 (55)[1]							13–128 (47.5)[1]
		80–95	55–95	75–80			60–80	75	60–80	60		
35		50–100	55–95	75–80		22–120	60–80	75	60–80	71 66		
36										90 83		
		45–110	60–105	75–80			60–80	75	60–80	101	53.9	
37											31.2 50.5	
		40–100	60–105	75–80		60–100	60–80	87	60–80	112	51.0	
38											27.3 35.6	
		60–110	60–105	75–80			60–80	80	60–80	100–127	53.1	
39											55.8 73.5	
		35–100	60–105	75–80		60–90	60–80	95	60–80	41 28–82	56.8	
40											47.0 71.6	
		35–100	60–105	75–80			60–80			25–112		

[1] Mean value.

In order to compare our results with those of other workers, reported in international units, a comparison of our serum standard with the international standard was determined by the method of Irwin (14). One arbitrary unit was found to be equivalent to 0.215 ± 0.016 international units by this method.

In actual practice, the unknowns were assayed on ten rats within the uterine weight ratio range of 0.8 to 2.0. This range was usually determined

by running a preliminary assay using three animals at each of two dose levels. The accuracy of the determinations, using ten animals and computing the results from the curve made with 20 animals at each dose level was calculated by the method of Irwin, and was found to be within 15 percent, when $P = 0.05$. As a further precaution we have considered as significant only those differences which are 25 percent or more.

SUMMARY OF NORMAL HORMONAL LEVELS IN PREGNANCY

The range of normal values for urinary pregnanediol excretion in late pregnancy found in the literature is reported in Table 3. From this table it may be seen that there is an increased excretion of pregnanediol as preg-

TABLE 4. URINARY EXCRETION OF ESTROGENS IN NORMAL PREGNANCY
(Values in mg./24 hr.)

Duration of pregnancy	Reference:	Browne Henry & Venning (7)	Lu (16)	Smith & Smith (26 & 27)			Hain (13)		Bachman (1)		
	No. of patients	8	87	15			4		6		
weeks	No. of observations	154	87	66			27		76		
				Estriol	Estrone	Estradiol	Estriol	Estrone	Estriol	Estrone	Estradiol
20		3–145									
28		7.5–23	0.4–1.3 (0.9)[1]								
29		7.5–23		8.5–10	0.5–0.9	0.07–0.14					
30		7.5–23									
31		7.5–23		9.5–10	0.3–0.4	0.06–0.11					
32		10–25	0.5–1.7 (1.04)[1]						14–	1.3–	0.3–
33		10–25		11–11.1	0.5–0.7	0.08–0.10			19–	2.2–	0.6–
34		10–25					0.7	0.5	10–18	1.0–2.3	0–0.15
35		10–25		12.5–13	0.2–0.8	0.05–0.14	1.4	0.8	9–19	0.5–2.3	0.2–0.4
							9.5	0.4			
36		15–40	0.6–3.6 (2.3)[1]				19.8	1.2	12–20	0.4–2.3	0–0.4
							14.8	1.9			
37		15–40		15.5–26.5	0.7–1.0	0.05–0.13	12.2	2.3	13–27	0.6–3.0	0–0.7
38		15–40					19.6	4.8	15–26	0.5–3.0	0–0.2
39		15–40		21.5–39.5	0.5–0.8	0.07–0.27	21.2	4.7–9	14–28	0.5–2.0	0–0.25
							24.5	5.3			
40		15–40	0.75–3.7 (2.5)[1]	10–11.5	0.4	0.15–0.7	4.2–19.3	0.85–1.9	11–25	1.0–2.5	0–0.4
							0.8–9.3				
41									15–26	1.0–2.1	0–0.7
42											

[1] Mean value.

nancy progresses, from 17–30 mg./day at week 20 to a peak of excretion in the eight month (weeks 32–35) with a range of values from 30–130 mg./day. There are variations in the rate of excretion of a somewhat cyclic nature. Within 24 hours of delivery the compound has practically disappeared from the urine.

TABLE 5. SERUM AND URINE LEVELS OF CHORIONIC GONADOTROPHIN IN NORMAL PREGNANCY
(Serum Values in International Units/100 cc.
Urine Values in International Units/24 hrs.)

Duration of pregnancy	Reference:	Jones (15)	Smith & Smith (21, 22, 23, 26, 27)	Smith & Smith (21, 22, 23)
	Number of patients	24	70	47
	Number of observations	133	263	140
weeks	Determinations on	Serum	Serum	Urine
20		450–750		
21		250–600		
22			1250–2500	10,000–30,000
23		270–1000	1250–2500	
24			1250–2500	6,750–50,000
25		87–800	1250–2500	
26		250–1500	1250–2500	10,000–30,000
27			1250–2500	
28		150–600	1250–2500	12,000–22,500
29		180–2500	1250–2500	
30		270–1400	1250–2500	
31		400–3000	1250–2500	
32			1250–2500	
33		250–1500	1250–2500	12,500–24,000
34		180–1200	1250–2500	
35		150–800	1000–5000	13,000–17,500
36		50–1500	1000–5000	
37			1000–5000	8,800–20,000
38		150–2000	1000–5000	
39		250–900	1000–7500	17,500–50,000

The urine estrogen values in late pregnancy which were reported in the literature in terms of mg./24 hours are recorded in Table 4. These values were determined by colorimetric procedures. If we disregard the values reported by Dr. Lu, which are not in agreement with those reported by the other workers, the pattern again shows an increase as pregnancy progresses with values from 10–15 mg. estrogen/day during the seventh and eighth months, and a rise to 15–40 mg./day in the ninth month. The curves for estriol, estrone, and estradiol exhibit parallel patterns. As with pregnanediol, the hormone disappears rapidly from the urine immediately following delivery.

In Table 5 are listed the normal serum and urine chorionic gonadotrophin levels as reported by Smith and Smith and by Jones and her co-workers. One rat unit of Smith and Smith is equivalent to 16.7–38 I.U.[1] In order to compare the levels found by Smith and Smith with those found by us and by Jones, we have multiplied their rat unit values by 25. It may be seen from this table that serum chorionic gonadotrophin in normal pregnancy maintains a fairly constant level, up to 3000 I.U., throughout the latter part of pregnancy, although a slight rise, as high as 5000 I.U. in the last month, and even 7500 I.U. just before term, is not abnormal. Urinary gonadotrophin excretion is also maintained at a fairly constant level, between 7000 and 30,000 I.U., with a possible rise to 50,000 I.U. just before term. This hormone also disappears rapidly from the serum and the urine immediately following delivery.

EXPERIMENTAL RESULTS

Simultaneous determinations of urinary estrogen and pregnanediol excretion and of serum gonadotrophin were made in five diabetic women throughout the latter part of pregnancy. In one case urinary gonadotrophin levels were also studied. The results of these studies, together with the clinical picture throughout pregnancy are presented in Tables 6 to 10. The hormone patterns in these patients are also presented in Figures 2 to 6.

Mrs. M. (Table 6, Figure 2) had no signs of toxemia and was delivered of a normal child. Her pregnanediol values varied between 54.2 and 82.8 mg. after the 28th week, which is in agreement with the normal values in Table 3. Estriol excretion varied between 7.7 mg. and 29.4 mg. after the 28th week, and estrone-estradiol between 0.4 mg. and 1.28 mg. These values agree with the normal values of the Smiths, Browne, Henry, and Venning, and Hain, but are higher than the values reported by Lu. Serum gonadotrophin, varying from 10,000 to 27,400 A.U., is in agreement with the normal values of the Smiths (1 A.U. =0.215 I.U.) but high when compared with the findings of Jones (15).

Mrs. DaL. (Table 7, Figure 3) who also showed no signs of toxemia, but whose baby died within a few hours after birth, showed pregnanediol excretion levels varying from 30.4 mg. to 77.5 mg. (mean of 50.9 mg.) after the 33rd week, which probably should be placed in the low normal range. Estriol excretion, varying from 1.61 mg. to 5.37 mg. is low according to all the investigators except Lu. Estrone-estradiol levels of 0.32 mg. to 0.75 mg. agree with the normal values reported by the Smiths and Hain. Serum chorionic gonadotrophin levels varying from 6750 A.U. to 20,600 A.U. were normal according to Smith and Smith, high according to Jones.

[1] Private communication.

TABLE 6. NORMAL PREGNANCY—CLINICAL AND HORMONAL DATA

Case 1—(Mrs. M.)

Date	Duration of pregnancy Weeks	Diet P	Diet F	Diet C	CLINICAL DATA: Daily insulin units	Urine sugar gm. 24-hr.	B.P.	Edema	Urine protein gm. 24-hr.	Wt. kg.	Wt. lbs.	Additional clinical data	Diab. status	URINE—values/24 hr.: Vol. cc.	Creatinine gm.	Pregnanediol mg.	Estrogens: Estriol mg.	Estrogens: Estrone-estradiol mg.	BLOOD: Gonadotrophin A.U./100 cc.
8/31/43	20	110	100	170	Regular 30-0-30	—	114/80	0	—	57.7	127			1800	—	13.7	1.5	0.15	—
9/18	22⅓				35-0-30	—	112/60	0	—	59.5	131	Upper respir. infec. 9/11–9/18		2000	—	35.4	3.8	<0.02	13,939
9/29	24				40-0-35	16	108/70	0	0.13	59.5	131		Good	1810	—	25.8	3.31	<0.02	11,050
10/12	26				40-0-35	42.0	120/70	0	0	61.8	136		Good	2100	—	20.9	3.32	0.44	14,111
10/26	28	100	100	220	48-0-38	27.4	112/70	0	spt.	63.2	139		Fair	1540	1.05	67.3	3.04	0.40	17,900
11/ 9	30				70-0-50	9.3	122/70	±	0.14	65.0	143	Edema of ankles on standing	Fair	1930	1.29	63.4	7.70	0.38	17,333
11/23	32				70-0-50-10	19.1	120/70	0	0.14	65.8	145	No edema	Good	1910	1.22	82.8	9.94	0.69	11,900
12/ 8	34				70-0-50	7.4	120/80	0	0.13	65.8	145		Good	1840	1.43	62.3	11.5	0.66	10,400
12/15	35				70-0-50	13.6	120/82	±	0.15	65.8	145		Good	2025	1.62	78.5	29.4	0.92	11,100
12/22	36				65-0-50	15.6	110/70/0	0	0.08	66.4	146		Good	2190	1.80	73.0	10.1	0.57	20,800
12/30	37				70-0-50	38.2	110/50/0	+	0.17	67.3	148	Edema of legs on standing	Good	2290	1.60	69.5	11.1	1.28	23,000
1/ 5/44	38	100	80	200	65-0-45	13.5	120/70	0	0.15				Good	1350	0.72	54.2	15.9	0.82	21,800
1/ 6					70-0-45	11.8	120/84	0	0.25				Good	1760	1.55	65.5	16.7	1.03	27,400
					Delivered by Caesarian section (1/6/44), 10:30 a.m.; baby weighed 3.6 kg.														
1/ 7					45-15-25	92.0	124/80	0	0.07				Good	920	1.34	6.9	1.14	0.10	4,800
1/ 8					35-10-15	11.5	120/80	0	spt.				Good	570	0.91	0	0.82	0.03	

TABLE 7. NON-TOXEMIC PREGNANCY—NEONATAL DEATH—CLINICAL AND HORMONAL DATA

Case 2—(Mrs. DeL.)

Date	Duration of pregnancy weeks	Diet			CLINICAL DATA									URINE—values/24 hr.					BLOOD
					Daily insulin units[1]	Urine sugar gm. 24-hr.	B.P.	Edema	Urine protein gm. 24-hr.	Wt. kg.	Wt. lbs.	Additional clinical data	Diab. status	Vol. cc.	Creatinine gm.	Pregnanediol mg.	Estrogens		Gonadotrophin A.U./100 cc.
		P	F	C													Estriol mg.	Estrone-estradiol mg.	
10/12/43	31	100	65	170	P 20 R25-0-10	33.0	110/70	0	0.14	54.6	120	Asymptomatic	Fair	660?	0.45	7.13?	1.22	0.22	9,270
10/26	33	120	65	180	P 20 R25-0-10	58.0	110/74	0	0.29	54.2	119	Brewer's Yeast, 22 gm. daily	Fair	1160	0.62	32.6	2.68	0.32	12,300
11/ 3	34				P 20 R20-0-10	6.9	118/70	0	0.14	55.0	121		Fair	620	0.76	30.4	1.61	0.39	7,570
11/ 9	35				P 20 R25-0-0	6.6	112/80	0	0.35	54.6	120	Dizzy spells; spots before eyes.	Good	985	0.73	57.5	3.98	0.75	6,750
11/17	36				P 20 R 25	20.7	122/80	±	0.41	—	—	Anorexia	Good	710 (11-19)	0.82	54.8	4.47	0.65	6,964
11/23	37				P 25 R 20	14.9	104/70	0	0.42	55.0	119	No symptons	Good	890	0.78	52.5	5.37	0.71	10,400
12/ 2	38				R15-10-15-10	15.7	100/78 132/100 100/72	—	0.59	—	—		Good	2035	1.30	77.5	2.56	0.49	20,600
		Delivered by Caesarian section (breech) (12/2/43), 12:02 p.m.; baby weighed 1830 gm.; expired 12/2/43; premature in appearance																	
12/ 3					R25-0-10	39.6	104/70	—	0.51	—	—		Good	2370	1.45	30.9	1.02	<0.02	5,040
12/ 4					R20-0-10	33.8	104/78	—	0.39	—	—		Good	1095	0.83	5.0	0.94	<0.01	—
12/ 5					R20-0-15	39.0	—	—	0.56	—	—		Good	1950	1.03	4.5	0.61	0.06	—
12/ 6					R35-15-15-10	—	116/80	—	—	—	—		Good	—	—	—	—	—	936

[1] "R" represents "regular" insulin.
"P" represents "protamine zinc" insulin.

TABLE 8. NON-TOXEMIC PREGNANCY—STILLBIRTH—CLINICAL AND HORMONAL DATA

Case 3—(Mrs. K.)

Date	Duration of pregnancy weeks	Diet	CLINICAL DATA								URINE—Values/24 hr.					BLOOD
		P F C	Daily insulin units	Urine sugar gm. 24-hr.	B.P.	Edema	Urine protein gm. 24-hr.	Wt. kg.	Wt. lbs.	Additional clinical data	Diab. status	Vol. cc.	Creatinine gm.	Pregnanediol mg.	Estrogens: Estriol mg.	Estrogens: Estrone-estradiol mg.	Gonadotrophin A.U./100 cc.
1/18/44	32+		P 15 R15-0-15	—	106/60	+	0	65.0	143	Prot. 5.60 A/G 3.52		1530	—	52.9	8.44	1.14	58,500
1/20	33		—	—	110/54	+	0	65.0	143			—	—	—	17.8	1.68	48,500
1/27	34		P 15 R20-0-15	30.2	110/80	++	0	66.3	146			2100	1.26	42.5	16.1	1.26	55,500
2/ 3	35		P 15 R20-0-15	56.3	118/80	++	0	67.7	149	Edema of legs		2250	1.42	60.8	16.9	1.56	45,800
2/10	36		P 15 R20-0-15	9.0	110/80	+	0	68.6	151			1750	1.23	40.2	—	0.15	44,500
			Fetal movement not felt 2/15/44														
2/17	37		P 15 R17-0-12	82.5	120/96	++	0	68.2	150			2500	1.48	53.8	<0.03	0.04	20,500
2/23	38		P 15 R17-0-12	12.1	110/70	0	0.2	—	—			1460	1.22	39.9	0.5	0.05	8,750
2/24			17-0-15-10	40.2	120/74	0	0	—	—			2590	1.45	61.5	0.25	0.01	—
2/25			20-0-20-10	40.0	110/74	0	0.2	—	—			1600	1.02	51.2	0.045	<0.01	10,200
2/25		8 hr. pre-labor		5.9	120/76	0	0.2	—	—			890	0.50	18.2	—	—	—
			Membranes ruptured artificially (2/26/44); macerated fetus														
2/26		18 hr.	15-0-10-10	33.6	—	0	—	—	—			2070	0.73	—	0.025	<0.01	3,870
2/27			P 15 R15-0-10	36.9	—	—	—	—	—			1840	1.43	—	0.06	<0.02	—
2/28			P 15 R15-0-10	23.4	—	—	—	—	—			1400	1.10	6.1	0.05	<0.01	900

TABLE 9. TOXEMIC PREGNANCY—VIABLE BABY—CLINICAL AND HORMONAL DATA

Case 4—(*Mrs. L.*)

Date	Duration of pregnancy weeks	Diet P	Diet F	Diet C	CLINICAL DATA: Daily insulin units	Urine sugar gm. 24-hr.	B.P.	Edema	Urine protein gm. 24-hr.	Wt. kg.	Wt. lbs.	Additional clinical data	Diabetic status	URINE (Values/24 hr.): Vol. cc.	Creatinine gm.	Pregnanediol mg.	Estrogens: Estriol mg.	Estrogens: Estrone-estradiol mg.	BLOOD: Gonadotrophin A.U./100 cc.
9/11/43	22	100	90	250	P 50 R20-0-15	—	120/64	0	—	60.4	133		Fair	2150	—	24.2	2.64	0.40	—
10/ 5	25				P 40 R20-0-20	48.5	116/80	0	0	65.8	145		Good	2680	—	20.5	1.38	<0.03	8,450
10/19	27				Same	72.3	112/80	0	0.21	—	—	Urticaria 10/18.	Good	2890	1.19	23.8	2.93	0.48	7,840
11/ 5	29⅓				P 40 R25-0-20	86.9	112/70	0	0.41	69.5	153	Pain left side of abdomen; no fever; Rx-bed-rest.	Poor	2840	1.20	27.5	—	1.03	8,583
11/15	31					54.6	—	—	0.25	70.6	155	Leg ulcer, in hospital.	Good	3520	1.70	39.8	11.3	0.44	6,375
11/23	32				P 40 R35-0-35	42.0	122/70	0	0.11	66.8	147	Ulcer healing.	Fair	2100	1.70	61.0	7.8	0.45	13,700
12/ 1	33				P 35 R40-0-35	38.6	122/72	0	0.15	69.5	153	Leg doing well; asymptomatic.	Fair	2040	1.30	38.9	5.36	0.66	14,000
12/ 7	34				P 35 R40-0-35	58.4	116/70	0	0.19	69.1	152	Leg not yet healed.	Fair	1770	0.64	5.4?	2.37	0.20	10,600
12/14	35				P 25 R25-0-35	8.3	124/70	0	0.13	70.6	155	Leg healed; asymptomatic.	Fair	1170	0.70	23.7	6.83	0.97	8,550
12/22	36				P 35 R40-0-35	5.6	126/72	0	0	71.8	158		Fair	1730	0.95	17.3	11.3	0.69	8,400
12/28	37				P 40 R35-0-35	24.4	130/80	+++	0.08	75.8	167	Edema 5 days; asymptomatic.	Fair	1220	0.77	17.0	7.17	0.24	10,600
12/30		100	60	180	R50-0-15-10	0	136/88	++	0.47	—	—	Bed-rest; edema less.	Good	4300	1.88	42.7	25.9	3.40	11,300
12/31		80	70	180	R40-0-10-10	0	120/86	+	0.34	—	—	Asymptomatic.	Good	2350	1.39	39.1	13.9	1.48	—
1/ 1/44					R30-0-10	0	120/80	+	0.15	—	—		Good	2100	1.53	52.2	14.1	1.42	—
1/ 2					R25-0-0	0	122/94	+	spt.	—	—		Fair	2640	1.60	50.9	18.9	1.42	—
1/ 3					R20-10-10-10	23.6	134/84	+	0.34	—	—		Fair	2360	1.51	55.7	17.9	1.14	—
1/ 4					R25-0-10-10	20.6	126/84	—	0.27	—	—		Fair	2470	1.43	—	11.6	0.91	—
1/ 5	38				R25-0-10	17.9	124.86	0	0.47	—	—		Fair	2160	1.51	50.8	13.9	1.09	16,600
					Delivered spontaneously (1/5/44) 2:40 p.m.; baby weighed 3.4 kg.; cystitis post-operative.														
1/ 6		100	80	180	R20-0-15-10	26.0	136.90	0	—	—	—		Fair	1560	2.50	—	2.46	0.46	5,080
1/ 7					R35-0-10-10	71.5	122/80	0	—	—	—		Fair	2860	1.95	12.8	1.45	0.4	—
1/ 8					R30-0-20-10	57.6	122/80	0	—	—	—		Fair	2830	2.49	—	0.97	<0.03	—

TABLE 10. TOXEMIC PREGNANCY—VIABLE BABY—CLINICAL AND HORMONAL DATA
Case 5—(Mrs. T.)

CLINICAL DATA													URINE—Values/24 hrs.							BLOOD
Date	Duration of pregnancy wk.	Diet			Daily insulin units	24 hour urine sugar gm.	B.P.	Edema	24 hour urine protein gm.	Weight in lbs.	Weight in kg.	Additional clinical data	Diabetic status	Volume cc.	Creatinine gm.	Pregnanediol mg.	Estriol mg.	Estrone-estradiol mg.	Gonadotrophin I.U.	Gonadotrophin A.U./100 cc.
		P	F	C																
9/11/43	26⅓	80	80	150	P50 R10	—	118/72	0	—	136	61.8		Good	3900	—	79.9	3.47	0.84	—	—
9/27	29				P60 R10	99.8	114/70	+	0.26	139	63.2	Gastric upsets	Poor	2400	—	65.6	4.0	0.45	32,600	51,400
10/13	31				P60	19.0	120/70	+++	0.19	145	65.9	U.R.I.[1] 10/1–10/12; nausea; diet intake low; Rx-Absolute bed-rest.	Poor	1420	0.84	75.7	5.82	0.36	33,200	48,600
10/19	32				P60	51	130/80	++	0.61	139	63.2	Nausea; bed-rest.	Poor	1890	1.06	53.2	4.57	0.29	62,500	63,750
10/25	33				P60	0	162/102	+	4.43	143	65.0	Headaches; nausea persists; diet intake poor; Rx-ethynyl testosterone stilbestrol and absolute bed-rest.[2]	Fair	2410	1.06	37.4	18.6	0.72	171,000	77,500
10/29					R35	0	170/110	++	4.49	145	65.9	Caesarian section (10-29-43) baby weighed 2500 gm.	Good	1920	1.02	60.7	7.57	0.60	167,000	59,400
10/31					R25	0	154/90	0	4.32	—			Good	5450	1.90	0	0.87	0.30	7,140	—
11/ 1					R20	—	150/90	0	—	—			Good	—	—	—	—	—	—	3,563
11/ 2					R40	64.7	134/78	0	3.02	—			Good	3350	1.32	0	—	—	6,750	—
11/ 3					R40	—	134/90	0	—	—			Good	2890	—	—	—	—	3,810	2,100

[1] Upper respiratory infection.
[2] 10–25–15 mg. ethynyl testosterone, and 20 mg. stilbestrol administered.
10–26 to 10–28–20 mg./day ethynyl testosterone, and 50 mg./day stilbestrol administered.

Mrs. K. (Table 8, Figure 4) who had no signs of toxemia, but whose baby died *in utero*, excreted normal amounts of pregnanediol (40.2–60.8 mg.) before fetal death, and this normal excretion continued for ten days after fetal death (39.9–61.5 mg.). Estriol levels of 16.1–17.8 mg., and estrone-estradiol levels of 1.14–1.68 mg. were normal until the time of fetal death,

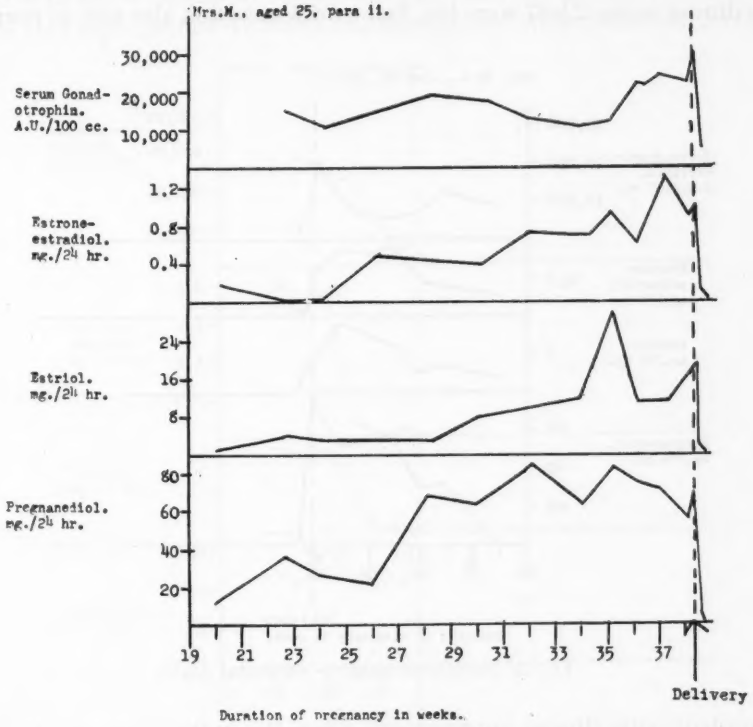

FIG. 2. Normal pregnancy.

after which they fell precipitously to values varying from 0.5 mg. to less than 0.01 mg. Serum gonadotrophin levels were markedly higher (44,500–58,500 A.U.) than the normals reported by other workers, and the level observed in our other non-toxemic patients, until the occurrence of intrauterine death. After fetal death the serum levels of chorionic gonadotrophin decreased (8,750–20,500 A.U.) but only to the levels which may be considered normal, and chorionic gonadotrophin did not disappear completely from the serum until after the surgical removal of the fetus and the placenta.

Mrs. L. (Table 9, Figure 5) who had mild symptoms of toxemia in the last week of pregnancy showed a continuous decrease in the amounts of pregnanediol excreted from 61 mg. at week 33 to 17.3 mg. at week 37, at which time symptoms of toxemia appeared. Pregnanediol excretion returned to normal (39.1–55.7 mg.) when bed rest therapy was instituted and the signs of toxemia decreased. Estriol excretion values of 1.38–11.3 mg. during weeks 22–37 were low, but estriol excretion also rose to normal

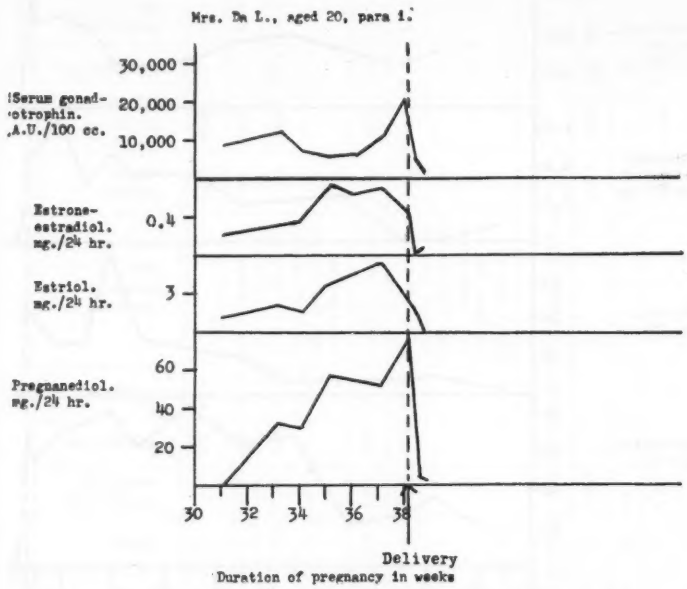

FIG. 3. Normal pregnancy—neonatal death.

coincident with clinical improvement when the patient was put to bed. Estrone-estradiol levels ranging from 0.24–3.40 mg. were normal throughout the period studied. Serum chorionic gonadotrophin levels varying from 6,375–14,000 A.U. were also normal.

Mrs. T. (Table 10, Figure 6) had severe toxemia with onset at 33 weeks. Pregnancy was terminated by Caesarian section a few days later. The amounts of pregnanediol excreted throughout the period studied were normal (53.2–79.9 mg.), showing a drop to one lower value (37.4 mg.) at the time of onset of the symptoms. It is not clear whether this value represents a significant drop or only a normal variation. When symptoms of toxemia appeared, the patient was put to bed and treated with ethynyl testosterone and stilbestrol; 15 mg. of pranone and 20 mg. of silbestrol on

the first day, and 20 mg. of ethynyl testosterone and 50 mg. of stilbestrol on each of the three succeeding days. Following this hormone therapy the pregnanediol excretion increased to 60.7 mg./day. Estriol values of 3.47–7.57 mg. were low, but one normal value of 18.6 mg. was found at the same time that the lowered pregnanediol value was determined. Estrone-estradiol from 0.29–0.72 mg. is normal throughout. Serum chorionic gonadotrophin levels were very high (48,600–77,500 A.U.) as compared with the normal levels of

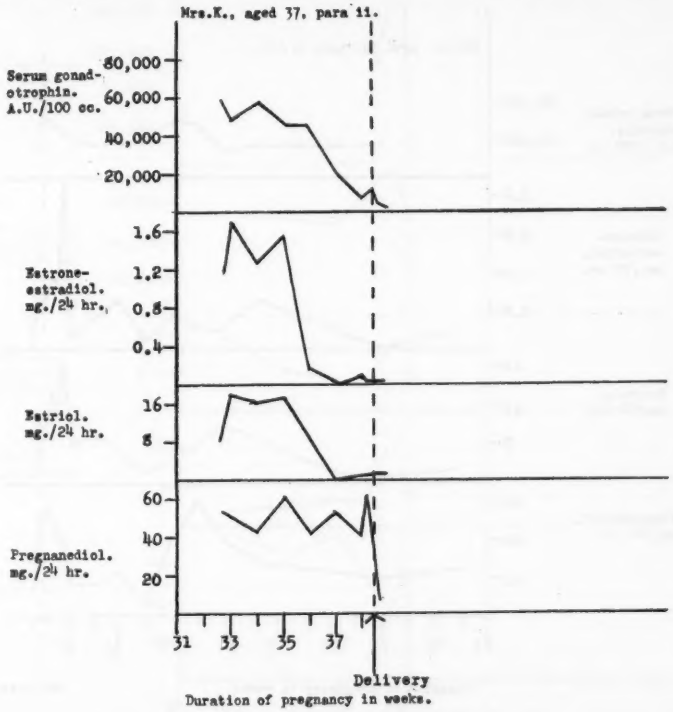

FIG. 4. Normal pregnancy—stillbirth.

the Smiths and of Jones, and with the levels found in *Mrs. M.*, *Mrs. DaL.*, and *Mrs. L.* There was no significant increase at the time of onset of toxemia. Urinary excretion of chorionic gonadotrophin was also studied in this patient. It is noticeable that at the time of onset of toxemia there was a very striking and significant increase in the urinary excretion of gonadotrophin, from 62,500 I.U. to 171,000 I.U., although the serum levels did not change significantly (63,750 A.U. to 77,500 A.U.). When compared with the normal urine levels of chorionic gonadotrophin reported by Smith and Smith, the level in this case was definitely increased.

DISCUSSION AND CONCLUSIONS

In a series of five pregnant diabetic women, three patients who had no signs of toxemia had normal levels of pregnanediol throughout the periods studied. One patient, with mild toxemia, had a low excretion of pregnanediol for several weeks. The patient with severe toxemia showed a relatively low excretion in one sample at the time of onset of toxemic symptoms. It is also of interest to note that pregnanediol excretion did not fall after intra-

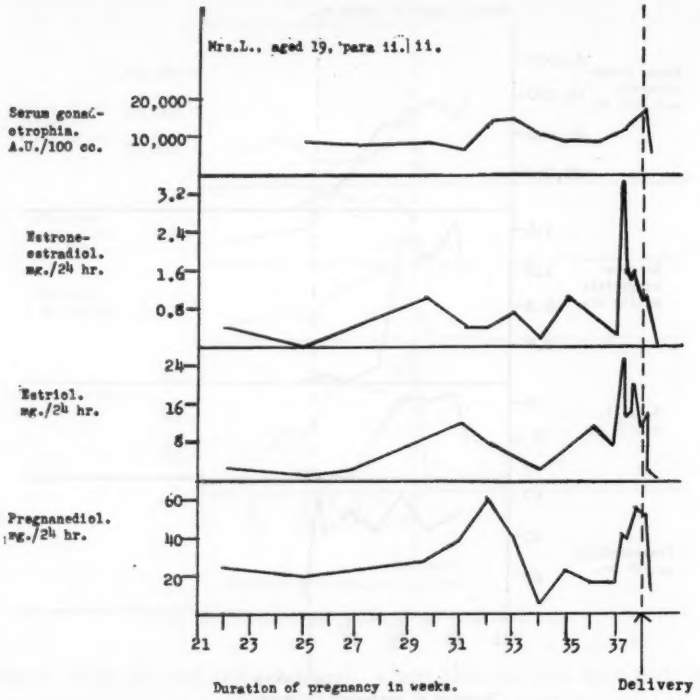

FIG. 5. Toxemic pregnancy—viable infant.

uterine death in one patient whose levels were normal before fetal death occurred.

Neither toxemia nor fetal mishap was found associated with any decreased excretion of estrone-estradiol, but a decreased excretion of estriol was found associated with both cases of toxemia, and with one of two cases of fetal mishap without toxemia.

In two of three patients who had no signs of toxemia the serum gonadotrophin levels were normal, and in the third patient the levels were significantly increased. Of two patients with clinical signs of toxemia, one patient with mild toxemia had normal levels of chorionic gonadotrophin and the

other, with severe toxemia, had significantly increased levels of gonadotrophin. Fetal mishap was associated with both normal and high levels of gonadotrophin.

A summary of the results of studies of pregnanediol and estrogen excretion and of serum chorionic gonadotrophin, together with the clinical data

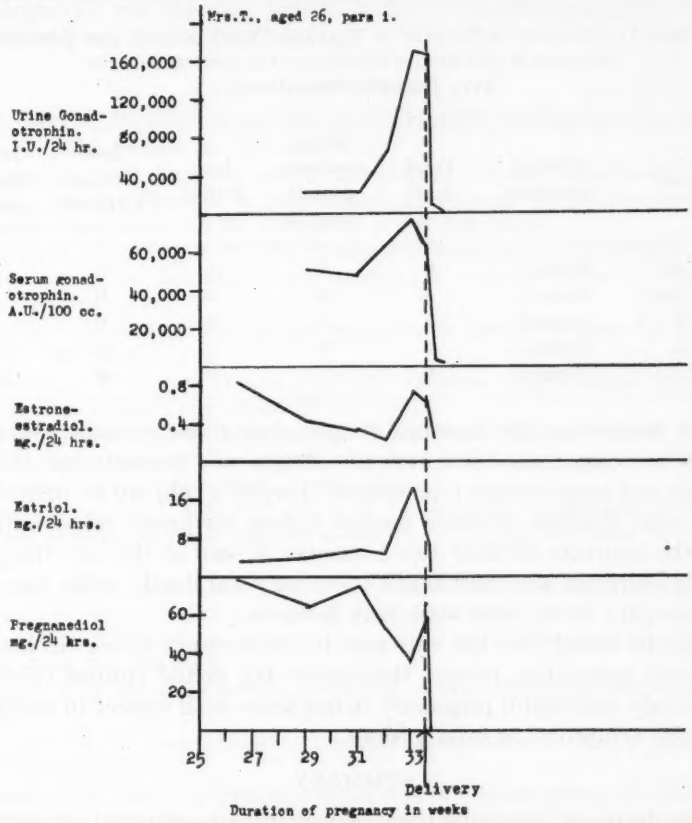

FIG. 6. Toxemic pregnancy—viable infant.

with respect to presence or absence of toxemia and fetal death may be seen in Table 11. From this summary it may be concluded that neither low estriol nor low pregnanediol excretion is consistently associated with fetal death, but that a lowered excretion of both appears to be associated with toxemia. Both fetal death and toxemia may or may not be associated with increased serum chorionic gonadotrophin. Hormone abnormalities were demonstrated in both of our patients with fetal mishap, but in one case the

abnormality consisted of an increased serum level of chorionic gonadotrophin and in the other case of a decreased excretion of estriol. White's theory that fetal death is associated with toxemia was not borne out by our small series (83).

Our finding of high serum chorionic gonadotrophin levels in a patient with normal estriol and normal pregnanediol excretion is not in keeping

TABLE 11. RESULTS OF STUDIES OF URINARY PREGNANEDIOL AND ESTROGEN EXCRETION AND SERUM CHORIONIC GONADOTROPHIN IN FIVE DIABETIC PREGNANCIES

Patient	Clinical condition	Fetal death	High chorionic gonadotrophin	Low estriol	Low estrone-estradiol	Low pregnanediol
Mrs. M.	Normal	0	0	0	0	0
Mrs. DaL.	Normal	+	0	+	0	0
Mrs. K.	Normal	+	+	0	0	0
Mrs. L.	Toxemic	0	0	+	0	+
Mrs. T.	Toxemic	0	+	+	0	+(?)

with the theory that the increased output of chorionic gonadotrophin is an attempt to compensate for a lack of estrogen and progesterone (15, 22). Estrogen and progesterone replacement therapy would not be indicated in such a case. Further evidence against a close reciprocal relationship between the amounts of these two hormones is seen in the fact that pregnanediol excretion was unchanged following fetal death, while the serum gonadotrophin levels were strikingly lowered.

It may be noted that the only case in our series in which the hormone levels were completely normal throughout the period studied (*Mrs. M.*) was the only uneventful pregnancy in our series with respect to occurrence of toxemic symptoms or fetal mishap.

SUMMARY

1. Simultaneous determinations of urinary estrogen and pregnanediol excretion and of serum gonadotrophin were made in five diabetic women throughout the latter part of pregnancy, and urinary gonadotrophin was studied in one. Two patients showed toxemia, and two who did not show toxemia suffered fetal mishap.

2. Low levels of estriol and pregnanediol excretion were found in both cases which showed toxemia, and low estriol was found in one non-toxemic patient whose baby died. Estrone-estradiol excretion was normal in all five cases.

3. Increased chorionic gonadotrophin levels were found in one patient

without toxemia, but with fetal mishap, and in one patient with toxemia, whose baby was normal.

4. Fetal mishap was not associated with toxemia in our small series.

5. Normal pregnanediol and estrogen values were found in a patient with high chorionic gonadotrophin.

6. The only patient in our series to show normal hormone levels throughout pregnancy was the only patient to have a normal pregnancy with respect to toxemia and to fetal mishap.

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RECURRENT DIABETES: SPONTANEOUS REMISSIONS AND EXACERBATIONS

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INTRODUCTION

"ONCE a diabetic always a diabetic" is a truism. Experience demonstrates that the diabetic may undergo either no changes, moderate (10) changes or tremendous (12) changes in carbohydrate tolerance. The seemingly mild case (2) may suddenly require large doses of insulin; normoglycemia is often maintained in the apparently severe case with successively smaller doses (21) of insulin, or even without it (11). A patient who has been diabetic for several years may even develop spontaneous hyperinsulinism (14). Recovery from diabetes has seemed to follow development of liver cirrhosis (1). Infrequently reports of cure (5) or apparent cure (8) of diabetes are found in the literature.

There is some reason to believe that cure is possible (3) in diabetes, but such claims are usually denied (13). This paper is not concerned with the cure that sometimes follows the correction of endocrinopathies (4) or of inflammatory processes (20) in the region of the pancreas.

As a result of inexplicable variations in carbohydrate tolerance, bizarre types (22) of treatment may appear equally as effective as the rational time-proven régimes. However, if it were established that there is a periodic factor operative in some persons with diabetes manifested by a spontaneous, inconstant, unpredictable and irregular tendency toward remission (19) the apparent improvement so induced would become understandable. The importance of treatment in accordance with time-proven physiologic principles (15) would not be questioned even though the tolerance of some patients might temporarily increase on a less rigid régime. All diabetics might benefit from better management of the physiologic abnormality characteristic of the disease. The physician's experience with an exceptional patient whose improved tolerance enabled him to consume large quantities of starch would not lead to unwarranted excesses in the management of the ordinary patient.

Of the patients continuously observed over a period of several years in the Jewish Hospital Clinic more than five per cent have shown irregular, inconstant, unpredictable and spontaneous remissions and exacerbations of the diabetic state. The remissions might be attributed to the therapeutic régime immediately preceding them. Likewise, the exacerbations might be

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attributed to the therapeutic régime immediately preceding them. But no novel treatment (either drug or dietary) was prescribed for the patients here reported. As a matter of fact essentially the same diet was prescribed for the individual patient as long as he was overweight whether a remission or an exacerbation was observed. It appears reasonable to conclude from the evidence available that the remission or improvement occurring in these cases was due to a natural inherent tendency toward remission present in some diabetics.

METHOD

A history with particular reference to the classical diabetic symptoms was taken on each patient. An oral glucose tolerance test (7) with 100 grams glucose was made, the content of sugar in the blood and urine being determined when the patient was fasting, and after one, two, three and four hours.

The obese patient was reduced whenever possible. The diet contained 60 to 80 gm. of protein, 150 gm. of carbohydrate or less, enough fat to meet caloric requirements. The diet of the ambulatory clinic patient is not always that prescribed. Efforts to ascertain the proximate composition of the diet of such patients have been unavailing, either through a written diary or through direct interrogation. Since the ambulatory patient often followed a diet of his own choosing rather than that prescribed, I am under no illusion that a particular diet played any part in the remission and exacerbations observed. *Cases 6* and *7* are particularly informative in this respect, the one readily admitting the consumption of large amounts of carbohydrate when not in the hospital, the other subsisting on a duodenal ulcer régime.

The aim of treatment in this series was to accomplish our usual objectives: to keep the weight normal, the urine sugar-free and the blood sugar normal. In the aglycosuric patient not taking insulin, a blood sugar estimation two to three hours after breakfast affords sufficient information as to the diurnal blood sugar level.

Glucose tolerance curves were repeated at intervals on those patients having a normal amount of sugar in the blood without insulin. When the repeated curve was normal in a patient not above his ideal weight, an unlimited diet except for the elimination of easily assimilable sugar (pastry, pie, candy, ice cream, etc.) was allowed as long as the weight remained stationary. In most instances this diet was not far different from that previously and actually consumed. Blood sugar determinations were made at intervals of approximately three months unless glycosuria recurred earlier. If the blood sugar was elevated a diet containing not more than 150 gm. of carbohydrate was again prescribed.

TOLERANCE CURVE INTERPRETATION

It is difficult to classify (16) every glucose tolerance curve. Such curves may be differentiated as diabetic, intermediate, normal, or non-diabetic with glycosuria. Since the present paper is not concerned with the latter condition no further mention is made of it. Nor is glycosuria recorded during the course of the tolerance tests. It is an extremely variable (6) phenomenon, appearing or not appearing more or less independently of the blood sugar level.

In an earlier paper (7) I expressed the opinion that if the blood sugar three hours after the ingestion of 100 grams glucose is less than 150 mg. per cent the patient does not have diabetes. Such a position is no longer tenable. If the maximum blood sugar value during the test occurs at the first hour and is not above 200 mg. per cent, the second hour's value is considerably lower, and the third is not above 120 mg. per cent, the curve is probably normal. If the maximum blood sugar level is above 200 mg. per cent, if there is a fall greater than 50 mg. per cent between the maximum level and that taken one hour later, and if the third hour's value is below 150 mg. per cent, the curve is intermediate. Otherwise it is diabetic. For clinical purposes an intermediate curve is equivalent to a diabetic curve.

However, there is no hard and fast rule in the interpretation of the test. Whenever the curve was abnormal, or there were symptoms suggestive of diabetes, the patient was kept under observation, and reduced if overweight. Blood sugar estimations after breakfast and repetition of the tolerance test served to establish the definitive diagnosis at a later date.

No special preliminary diet preceded the glucose tolerance test although this is the custom of some investigators. Occasional tests on hospitalized patients after several days of isocaloric diets, the one high in carbohydrate, the other high in fat, revealed no difference in the curve which would contribute to the differential diagnosis. This is strikingly illustrated by the last two curves on *Patient 6*. In this instance the curve after the high fat diet is appreciably lower than that after the high carbohydrate diet although both are normal. I am well aware of the several reports which indicate that previous diet exerts a considerable influence on the form of the glucose tolerance curve.

In the tables, **bold face type indicates a curve interpreted as normal;** *italics, a curve interpreted as intermediate;* regular type, a curve interpreted as diabetic.

The pertinent data for the first seven patients are briefly presented in Tables 1 to 7. Three other cases, similar to the above have been studied.

DISCUSSION

The irregular, inconstant, unpredictable and spontaneous fluctuations in the tolerance of the diabetic persons here reported substantiate a single fact: there are unexplained variations in individual response to the ingestion of 100 grams of glucose. Except as indicated occasionally there were no discoverable intercurrent pathologic or physiologic processes which might have altered the response. The greatest variation in relatively inert body mass (*i.e.*, fat) amounted to 23 per cent (*Case 3*); the least, 10 per

TABLE 1. SUCCESSIVE GLUCOSE TOLERANCE CURVES IN CASE 1

Date	Classification	Fasting	After 1 hr.	2 hr.	3 hr.	4 hr.	Weight kg.
		True blood sugar in mg. per cc.					
Dec. 15, 1930	D	115	194		181		88
June 29, 1933	**N**	**87**	**127**	**143**	**109**	**89**	71
May 15, 1934	**N**	**80**	**128**	**100**	**75**		72
Dec. 14, 1934	**N**	**85**	**163**	**154**	**114**		77
Nov. 30, 1935	**N**	**88**	**141**	**73**	**55**	**67**	77
June 13, 1936	**N**	**87**	**163**	**116**	**92**	**50**	77
April 17, 1937	**N**	**94**	**120**	**79**	**50**	**67**	79
Jan. 21, 1938	*I*	*98*	*194*	*177*	*127*	*67*	79
Aug. 27, 1938	D	116	227	272	210	197	74
(With cellulitis of foot—after two days on P70 F50 C250)							
Oct. 29, 1938	N	**107**	**222**	**188**	**102**	**61**	79
March 13, 1939	D	145	247	279	250	184	72
(After three days of fever and ?catarrhal jaundice)							
March 24, 1939	D	112	240	252	181	109	72

Male (*J.H.D. 838*) aged 66 when first seen in 1930 following the accidental discovery of diabetes; his weight was 76 kilograms. Death, presumably due to a carcinoma of the head of the pancreas occurred in September, 1939.

In this, as in the succeeding tables, **bold face type indicates a curve interpreted as normal**; *italics, a curve interpreted as intermediate,* regular type, a curve interpreted as diabetic.

cent (*Case 2*). Yet body mass is not the only factor operative in the change from diabetic to intermediate to normal response to the glucose tolerance test. The other important factor is probably the functional capacity of the beta islet cells. Variations in absorption of glucose in the test may occur, but are probably only minor in degree and could not account for other corresponding clinical evidences of changes in tolerance.

Case 1, after the loss of 17 kilograms of fat, showed a succession of normal curves which continued to be normal despite the redeposition of almost half the lost weight. At the same increased weight (result of a freely chosen rather than the prescribed diet), an intermediate curve developed.

Hence the excess of body fat is not the only factor operative in the redevelopment of a diabetic curve. The infectious (febrile jaundice) as well as the degenerative (pancreatic carcinoma) process, each activated an unknown diabetogenic factor at a time when the patient weighed much less than when his curves were normal. But the unknown diabetogenic factor was already operative in January, 1938, (as it had last been in December, 1930) when there was no discernible complication. It may be assumed that the homeostatic mechanism of the body was sufficient to neutralize the diabetogenic factor over a period of five and a half years.

TABLE 2. SUCCESSIVE GLUCOSE TOLERANCE CURVES IN CASE 2

Date	Classification	Fasting	After 1 hr.	2 hr.	3 hr.	4 hr.	Weight kg.
		True blood sugar in mg. per cc.					
Oct. 14, 1931	D	105	180	207	181	145	60
Jan. 30, 1932	D	96	271	199	170	156	54
Oct. 27, 1933	D	112	241	221	154		61
Mar. 15, 1935	D	116	258	224	158	102	59
Mar. 21, 1936	*I*	*109*	*257*	*207*	*135*	*106*	60
Mar. 5, 1937	*I*	*103*	*240*	*213*	*126*	*110*	58
June 18, 1937	*I*	*115*	*235*	*106*	*138*	*127*	58
Jan. 28, 1938	**N**	**97**	**234**	**160**	**128**	**121**	59
Dec. 9, 1938	**N**	**99**	**165**	**122**	**112**	**116**	57
Nov. 25, 1939	**N**	**92**	**196**	**122**	**90**	**72**	59
Dec. 27, 1940	D	162	298	330	298	280	59

Female (*J.H.D. 1883*), aged 43 when first seen in 1931 having classical diabetic symptoms of two weeks duration; her ideal weight was 60 kilograms. During nine years of observation her weight was within the normal limit of variation.

Case 2 presented a succession of diabetic curves over a period of six years; then, without appreciable change in body weight, an intermediate curve was obtained. The second curve of 1938 and the 1939 curves were normal. Had any therapeutic novelty been introduced during the preceding months, the improvement would have been attributed to it, but there had been no change in the therapeutic régime. Still without change in the therapeutic régime, a curve was diabetic 13 months after the second normal curve. There had been no change in either the body weight or the diet prescription. The only tenable conclusion is that from time to time the individual's response to the standard glucose tolerance test varies independently of presently discoverable causes. Perhaps the tendency to revert toward the normal is another manifestation of the homeostatic mechanism of the body. This, in turn, seems finally to be offset by the disease process. The ultimate factor in the determination of clinical dia-

betes is the functional capacity of the islet *beta* cells. The mild course of the disease in this patient over a nine year period resulted from the inherent power of the body to preserve their function, at least temporarily. It is not unlikely that a régime calculated to prevent glycosuria and to preserve normoglycemia acted as a powerful aid in this direction. Even so, finally, the diabetogenic factor prevailed over the homeostatic mechanism, as manifested by the elevated fasting blood sugar at the time of the last glucose tolerance curve. Had the diet been excessively high in carbohydrate content it is not unlikely that a diabetic curve would have recurred much sooner.

Case 3 included two diabetic curves followed by a normal, an inter-

TABLE 3. SUCCESSIVE GLUCOSE TOLERANCE CURVES IN CASE 3

Date	Classification	Fasting	After 1 hr.	2 hr.	3 hr.	4 hr.	Weight kg.
		True blood sugar in mg. per cc.					
Dec. 15, 1934	D	121	265	197	141		78
Mar. 11, 1935	*I*	*91*	*211*	*225*	*137*	*62*	63
Jan. 10, 1936	**N**	**95**	**197**	**140**	**88**	**56**	64
Aug. 14, 1936	*I*	*97*	*185*	*176*	*130*	*124*	63
Aug. 13, 1937	**N**	**88**	**162**	**119**	**121**	**63**	62
July 16, 1938	*I*	*87*	*200*	*153*	*120*	*79*	67
June 24, 1939	**N**	**85**	**145**	**129**	**113**	**74**	60
Aug. 8, 1940	**N**	**98**	**171**	**134**	**105**	**67**	64

Female, (*J.H.D. 2504*), aged 51 when first seen in December, 1934, because of symptoms due to an elevation of the blood sugar; her ideal weight was 58 kilograms.

mediate, and some years later, two normal responses. The primary improvement seems related to the loss in body weight. However, the first intermediate curve was obtained without significant further weight change. The second intermediate curve was preceded by a weight gain of only seven per cent, hardly enough to exert an appreciable effect; or is it? Further weight reduction was accompanied by a normal curve. It might be argued that the change in tolerance was the result of a lesser body mass. Or that it was evidence of the body's effort to maintain homeostasis at the expense of the diabetogenic factor, first the one, than the other being in the ascendant. The ultimate, determining factor, of course, is the functional integrity of the islet *beta* cells. Their capacity to produce insulin evidently varies from time to time. Possibly, even though the weight is unchanged, the next curve will be diabetic. There is no reason to assume that the diet prescribed played any part in these spontaneous exacerbations and remissions.

Case 4 resembles *Case 3* in its recurring succession of diabetic, intermediate and normal responses to 100 grams of glucose. Body weight may play a part in this variation, but at a weight of 63 kilograms, both a normal and a diabetic curve were obtained; and at 64 kilograms, an intermediate curve. Later, at a weight of 54 kilograms, the curve was intermediate, while at 63 as well as 54 kilograms, it was normal. Had the patient been treated by a therapeutic novelty during any one of several intervals, spectacular improvement would have been attributed to it. But no

TABLE 4. SUCCESSIVE GLUCOSE TOLERANCE CURVES IN CASE 4

Date	Classification	Fasting	After 1 hr.	2 hr.	3 hr.	4 hr.	Weight kg.
		True blood sugar in mg. per cc.					
July 14, 1934	D	128	280	286	250		64
Mar. 7, 1935	D	82	197	101	150		54
Aug. 30, 1935	**N**	**89**	**120**	**107**	**98**	**47**	55
Feb. 29, 1936	*I*	*99*	*198*	*147*	*138*	*63*	59
Aug. 14, 1936	*I*	*103*	*228*	*186*	*137*	*112*	61
Feb. 2, 1937	**N**	**99**	**195**	**149**	**122**	**63**	63
Jan. 29, 1938	D	99	216	224	213	141	63
Oct. 21, 1938	**N**	**123**	**240**	**178**	**97**	**58**	64
Sept. 29, 1939	D	106	220	180	144	92	63
Aug. 29, 1940	**N**	*89*	*165*	*98*	*134*	*94*	54

Female (*J.H.D. 4206*), aged 57 when first seen in 1934 having classical diabetic symptoms of several weeks duration; her ideal weight was 54 kilograms. Twenty units of regular insulin daily were required to maintain normoglycemia in the early period of treatment.

novel therapy had ever been prescribed, either in the form of drug or diet. The marked fluctuations in weight are ample evidence that the patient did not rigorously follow the prescribed diet. Indeed, they emphasize the conclusion that neither diet nor weight alone determine the presence of a normal or a diabetic curve. This patient, as do many others, illustrates the difficulty of making a definitive diagnosis of diabetes on the basis of individual glucose tolerance tests.

Case 5 at first glance seems to afford an illustration of an improvement in glucose tolerance actuated by weight loss. But without weight gain the curve became diabetic. Since it was obtained six weeks after the onset of the coronary occlusion and after five weeks without fever the occlusion probably played no part in the reversion. The coincident, prolonged, bed rest may have descreased glucose tolerance through the withdrawal of the effects of muscle activity. Exercise increases the ability of the diabetic to

TABLE 5. SUCCESSIVE GLUCOSE TOLERANCE CURVES IN CASE 5

Date	Classification	Fasting	After 1 hr.	2 hr.	3 hr.	4 hr.	Weight kg.
April, 1936		After breakfast blood sugar levels: 195, 167					70
July 31, 1936	N	95	116	127	141	94	62
Feb. 20, 1937	N	93	178	156	92	45	62
Aug. 20, 1937	N	84	168	158	97	88	64
Oct. 13, 1939	D	79	166	206	167	138	61
		(six weeks after coronary occlusion)					
July 9, 1941[1]	N	67	142	121	115	62	67

Male (*J.H.D. 15774*), aged 52 when first seen in 1936 because of mild diabetic symptoms; his ideal weight was 63 kilograms.

[1] Data supplied through the courtesy of Dr. S. D. Katz.

utilize sugar. With unrestricted activity, nearly two years later the curve was normal despite an appreciable weight gain and the admitted consumption of interdicted foods. The later course of the patient proves that weight is not the sole factor in the change from a diabetic to a normal curve and even that diet may be relatively unimportant. Nevertheless, it is my clinical impression that the consumption of excessive amounts of carbohydrate, while not the sole factor, contributes to an earlier breakdown of the homeostatic mechanism in some diabetic persons.

Case 6 presents a curious set of observations which emphasize the paucity of our knowledge in regard to some diabetogenic factors. The patient

TABLE 6. SUCCESSIVE GLUCOSE TOLERANCE CURVES IN CASE 6

Date	Classification	Fasting	After 1 hr.	2 hr.	3 hr.	4 hr.	Weight kg.
		True blood sugar in mg. per cc.					
March 2, 1935		422	3				74
July 13, 1938	D	216	372	438	430	332	70
		(Left leg amputated August, 1939)					
		(Right leg amputated November, 1940)					
Jan. 2, 1941	N	92	176	173	117	88	
		(After 4 months on diet of P 70, F 80, C 200—1900 Cal.)					
Jan. 13, 1941	N	88	126	147	91	37	
		(After 10 days on diet of P 70, F 135, C 75—1795 Cal.)					
July 25, 1941	D	155	(without dehydration)				

Male (*J.H.D. 14667*), aged 40 when first seen in 1935 with classical diabetic symptoms of 18 months' duration; his ideal weight was 64 kilograms. As much as 100 units of protamine insulin were required daily to maintain normoglycemia. No insulin was required for four months preceding the glucose tolerance curves made in January, 1941. Death followed cerebral hemorrhage in July, 1941.

required large doses of insulin during the early period of treatment. Later, and after four months without this adjuvant, he showed two normal glucose tolerance curves. The failure of isocaloric high carbohydrate and high fat diets to produce a difference in the glucose tolerance curve is noteworthy.

The interpretation to be placed upon the reversion of an obviously severe diabetic to normal glucose tolerance is not clear. It probably does not spring from the reduction in body mass for the greater portion of the legs, being muscular, are not inert. In the other cases the reduction in body mass was all at the expense of relatively inert fat tissue. In them it might be supposed that the total, absolute decrease in metabolic load was responsible for the improved tolerance. In this patient the decrease in metabolic load was only relative, for the larger part of the surgically removed tissue was actively functional. Furthermore, there had been a decided improvement in carbohydrate tolerance prior to the first amputation, since during hospitalization in July, 1939, only 10 units of protamine insulin maintained normal blood sugar levels. Even before amputation of the second leg normal blood sugar levels were maintained without insulin. This unusual case emphasizes the variability in the severity of diabetes and that this variability, which arises in the changing functional capacity of islet *beta* cells, is not directly and solely dependent upon either body weight or the character of the food ingested.

The validity of this conclusion is strengthened by the fact that during the six months at home preceding his death there were no symptoms of diabetes. He consumed, according to his diabetic landlady, large amounts of bread and other starchy foods. The absence of legs forced inactivity upon him. Whether the lack of exercise, or the very excessive carbohydrate intake, or some other diabetogenic factor ultimately overcame the homeostatic mechanism of the body cannot be determined from the available evidence. The elevated fasting blood sugar without hemoconcentration four days before death permits no doubt that the diabetes had recurred. A single factor cannot be incriminated as causative.

Proponents of extremely high carbohydrate diets would have reason for elation over the results of treatment as reflected in the glucose tolerance curves made in January, 1941. They might, however, need to pause before offering an explanation for the recurrence of the diabetes. It might be wiser to accept the view that all diabetics, given time and opportunity and despite anything that may be done, will ultimately again show unmistakable evidence of the disease even though there may be periods during which every test discloses a normal glucose metabolism.

Case 7 presents the record of a patient in whom diabetes was accidentally discovered in 1929. She has never been treated for this, but has been

observed at intervals. There is great variability in her weight and in the form of her glucose tolerance curves, but on the whole the latter are diabetic. It is a fair presumption that the excessive carbohydrate intake (including large amounts of easily assimilable sugar) acted as an exhausting agent on islet *beta* cell capacity despite a strong homeostatic effort on the part of the body for ten years. It was not until 1939 that there was appreciable elevation of the fasting blood sugar level. In the meantime, the exhaustion of beta cell function induced jointly by excessive body weight, very excessive carbohydrate intake, perhaps even diminished physical

TABLE 7. SUCCESSIVE GLUCOSE TOLERANCE CURVES IN CASE 7

Date	Classification	Fasting	After 1 hr.	2 hr.	3 hr.	4 hr.	Weight kg.
		True blood sugar in mg. per cc.					
May 11, 1929	D	112	299	180	126		57
May 22, 1930	*I*	*117*	*231*		*58*		69
Oct. 3, 1931	*I*	*113*	*194*	*150*	*135*	*75*	59
June 28, 1934	D	120	252	219	163		64
Dec. 29, 1934	*I*	*116*	*252*	*120*	*90*		60
Oct. 12, 1935	**N**	**116**	**194**	**123**	**100**	**50**	60
Sept. 10, 1937	D	119	208	192	154	104	56
Dec. 15, 1939	D	151	276	215	125	88	70
Jan. 7, 1941	D	184	313	243	182	138	67

Female (*J.H.D. 2997*), aged 31 when first seen in 1929 on account of a duodenal ulcer; her ideal weight was 60 kilograms. She was treated by the gastro-enterologist with a high carbohydrate diet; she tended toward obesity, and developed hypertension and coronary disease.

activity and the failure of the homeostatic mechanism finally resulted in a distinctly diabetic glucose tolerance curve. It is certainly possible that had the weight been normal or the starch consumption less the fasting blood sugar values would have remained normal. The combination of diabetogenic factors was too great for the homeostatic mechanism to overcome. Such an aggravation of diabetogenic factors may be overlooked by some of the proponenents of excessive carbohydrate intake as a treatment for diabetes. Their zeal may be aroused by such a temporary regression as shown in 1935 by this patient. Prolonged, careful observation should dampen such zeal, since temporary improvement may follow a change to a higher carbohydrate diet, but eventually loss of tolerance is the rule (18).

It is strongly emphasized that gains in tolerance are more commonly to be observed after restricting rather than after allowing very high carbohydrate intake. MacBryde (17) has presented evidence which indicates

that by clinical studies and insulin tolerance tests it is possible to predict which diabetic will respond best to each type of diet. In general, the young insulin-sensitive patient tolerates high carbohydrate régimes poorly; such patients are apparently primarily pancreatic (insular) diabetics. Older persons, often obese, frequently show at least temporary improvement after high carbohydrate allowances; their diabetes may be largely extra-insular (? hepatic, etc.) and it is relatively insulin-resistant (17).

If the glucose tolerance curve reverts to normal, even temporarily, it indicates a tremendous improvement in ability to utilize glucose. Presumably it indicates greater functional capacity of the islet cells and less hydropic degeneration. Since the patient whose weight is being reduced is actually on a high fat diet (consuming body fat) these experiences may contribute clinical confirmation to the significant animal experiments of Haist, Campbell and Best (9), showing that low carbohydrate, or high fat diets or fasting improve tolerance in pancreatic diabetes.

The glucose tolerance test affords no indication of the severity of diabetes. Nevertheless it does afford a rough index of the quantity of insulin produced and of its effectiveness. If the product of these two factors (quantity and effectiveness) is high following the administration of glucose, the peak of the curve is low. If the product is somewhat less the curve may be high but if later, insulin production is accelerated, there is a precipitous fall in the blood sugar level. If insulin production is not accelerated the blood sugar remains high and the curve is clearly diabetic. This is the reason for distinguishing an intermediate curve. Presumably it indicates a degree of exhaustion of beta islet cell capacity less than that in the patient showing a diabetic curve. I am aware of the objections (16) raised against the glucose tolerance curve and that the proposition here outlined is theoretic. Nevertheless it appears as a useful interpretation of the observed results.

SUMMARY

1. Ten patients were observed over periods of from two to twelve years, each showing diabetic, intermediate and normal responses to the standard glucose tolerance test at various times.

2. The reversion of a diabetic tolerance to normal is not infrequent since these patients constitute at least five per cent of the diabetic patients regularly reporting to one out-patient clinic for several years.

3. Patients whose curves reverted to normal did not always continue to show the normal response, there being considerable variation in the type of curve.

4. There appeared to be some evidence of a relationship between body weight (relatively inert fat) and the type of curve. In general, the more

closely the weight approached the ideal the greater the tendency for the appearance of a normal tolerance curve. Frequent exceptions to this generality are emphasized.

5. It is suggested that there is a spontaneous remission and exacerbation in some diabetic persons, that diabetes tends to appear and disappear in a small percentage of cases as judged by ordinary clinical methods which are crude measures of islet *beta* cell capacity. It is suggested that this unpredictable variation may be a manifestation of the homeostatic mechanism of the body.

6. It is suggested that in the interpretation placed upon any "new" treatment of diabetes (particularly if it is not rooted in the sound physiology of both old and recent research) due account be taken of the factor of unpredictable variation.

CONCLUSION

The person posssessed of the capacity to develop diabetes retains the potentiality in spite of apparent cure. Various pathologic proceses, some discernible, some not, may reactivate it. There is a variability in diabetes which manifests itself in some patients through irregular, inconstant, unpredictable and spontaneous fluctuations in tolerance in the form of remissions and exacerbetions. Reduction to the ideal weight and a diet which prevents glycosuria and maintains normal blood sugar levels appear to take the greatest possible advantage of this inherent homeostatic mechanism. But this is not invariably and necessarily true. The observations here recorded cast some doubt on the validity of the conclusion of some workers that patients have gained tolerance solely as a result of excessively high carbohydrate diets. The truism, "once a diabetic always a diabetic," is not overthrown because some patients manifest no clinical or laboratory evidence of diabetes over a period of several years. Such patients, given time and opportunity, will again develop unmistakable evidence of diabetes.

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THE INFLUENCE OF VARIOUS ANDROGENIC STEROIDS ON NITROGEN BALANCE AND GROWTH[1]

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IT HAS been demonstrated by a number of workers (1, 3–5, 7, 8, 10–14, 20, 21) that testosterone propionate causes a retention of nitrogen and other tissue-building elements in human beings, thus confirming older observations of Kochakian (15) on castrate dogs. Methyl testosterone administered orally has similar effects (6, 13, 20, 22, 24). The anabolic effects of the androgens have been reviewed comprehensively by Kenyon, Knowlton and Sandiford (9).

When a patient is given a constant nitrogen intake, the administration of testosterone propionate or methyl testosterone causes a decrease in the urinary nitrogen and no appreciable change in fecal nitrogen, so that increased amounts of nitrogen are retained. Inorganic phosphorus and sulphur in the urine are similarly affected by testosterone propionate. Phosphorus is retained approximately in the ratio to nitrogen of 1:10. The ratio of nitrogen to sulphur estimated as retained often approximates the normal proportion of these elements in body protein. These changes are often apparent by the second or third day and reach their maximum between the fifth and fifteenth days. During treatment with androgen there is a rapid gain in body weight which is partly accounted for by the building of protein tissues and partly by the retention of fluid. When androgen is discontinued, the output of nitrogen increases temporarily to a higher level than before treatment, indicating that there is at least a partial loss of the amount retained. There is also a decrease in weight. In children there is an acceleration of the rate of growth during treatment.

These observations suggested the possible therapeutic value of using androgen to promote protein anabolism in conditions of debilitation, in retarded growth accompanied by sexual infantilism, in Cushing's syndrome and perhaps in other disorders. We (24) have used methyl testosterone and

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testosterone propionate to stimulate the growth of sexually immature dwarfs who had reached an age when the persistence of sexual infantilism suggested a generalized pituitary deficiency. In a few dwarfed girls believed to have pituitary infantilism we have employed combined treatment with female sex hormones and methyl testosterone with gratifying results.

It was obvious that if substances could be found which had strong protein-anabolic, growth-stimulating effects but caused little or no virilization they would have even broader therapeutic applications, especially in the treatment of young children or of females. The possibility that some of the steroids having chemical structures related to testosterone might fulfill these specifications occurred simultaneously to a number of different investigators who made clinical studies of their effects on the nitrogen metabolism. These data were assembled by Reifenstein (18) and reported to the *Conference on the Metabolic Aspects of Convalescence and Wound Healing* at the meeting in December, 1942. Although different workers had employed somewhat different technics of study and different types of cases, Reifenstein attempted to analyze the data and evaluate the anabolic effects of the various steroids in terms of the amount of nitrogen retained daily per kg. of body weight. At the same time, Kochakian (16) investigated a large number of steroids by comparing their effects upon the growth of the seminal vesicles and prostate and upon the growth of the kidney of the rat, in the belief that the growth of the kidney might to some extent parallel the general anabolic effects.

METHODS

We had previously studied the effects of methyl testosterone and testosterone propionate on the nitrogen balance and creatine excretion in a number of sexually immature children who were stunted in growth. The effects were the same as those observed by others in the treatment of adults. In addition the children showed a marked acceleration in the rate of growth and increased muscular development similar to that which occurs in normal adolescence. It seemed that immature children would be especially suitable for the study of the protein-anabolic effects of other weaker androgens because such patients produce but little endogenous androgen and might presumably be especially sensitive to the substances administered.

Our studies were necessarily limited to those steroids of the androstene and androstane series exhibiting negligible virilizing effects, which could be made available in sufficient amounts for clinical trial. It was our plan to study the effect on the nitrogen retention of one or more of these compounds on each patient and then to compare the effect of testosterone propionate, methyl testosterone or unconjugated (free) testos-

terone on the same patient. Periods of treatment varied from 15 to 45 days according to the amount of material available for study. Between each period of treatment there was a control period of 10 days or longer.

Each patient was kept on a constant nitrogen intake throughout the study and the nitrogen balance was determined before treatment was begun, and throughout the subsequent periods of treatment and the intervening control periods. Since we were studying at the same time the excretion of creatine, meat and fish were excluded from the diets although milk, cheese and eggs were given. A survey of the quality of the protein indicated that there was no deficiency of any of the amino acids. Carbohydrate and fat were apportioned in the ratio of 3 gm. of carbohydrate to 1 gm. of fat. That the diets were adequate is shown by the fact that all the patients except *No. 8* and *No. 9* showed marked nitrogen retention and weight gain when treated with testosterone propionate or methyl testosterone. After the diet was adjusted during the preliminary control period, it was usually continued unchanged throughout the subsequent studies. However, in several instances increasing hunger made it necessary to increase the caloric content although the protein was kept constant.

All studies were made on a special metabolism ward. Twenty-four hour specimens of urine were analyzed daily for total nitrogen, creatine and creatinine. The diets were not analyzed but their composition was calculated from standard food tables. Analyses of the feces were made in only three cases before and during treatment with methyl testosterone. In these cases the fecal nitrogen was regularly between 0.3 and 0.5 gm. per day and did not change significantly during treatment. However, in calculating nitrogen balances, in order to conform with other workers, we have considered the fecal nitrogen as 10% of the nitrogen intake. Since the fecal nitrogen does not change appreciably with treatment, the validity of this assumption does not affect the estimation of the nitrogen retention which is caused by a particular steroid.

The charts showing the nitrogen balance studies are constructed along the lines used by Albright and the Boston group of workers. To avoid the confusion introduced by small daily fluctuations, each column represents an average for a 5-day period. The distance between the base line and the bottom of the shaded columns shows the total nitrogen intake. The length of the shaded column indicates the amount of the urinary and fecal nitrogen. The distance between the top of the shaded column and the base line shows the nitrogen balance, which is positive if below the base line and negative if above.

The studies reported were made on 12 male patients between the ages of 8 and 18 years who are listed in Table 1. Ten of them showed delayed growth and osseous development but were in good health and were not

TABLE 1

Case no.	Name	Age yr.	Height		Weight kg.	Bone age yr.	17-K.S. mg./24 hr.	Diet		N-balance before treatment gm. per day
			in.	cm.				prot. gm./kg.	cal./kg.	
1	*W.L.*	15	56.4	143.3	28	11½	2.5	1.18	45.3	+0.11
2	*L.D.*	15½	50.0	126.0	29	10½	2.5	1.88	56.8	+1.36 +0.21
3	*N.W.*	17	55.4	140.6	42	11½	3.9	1.72	60.8	+0.97
4	*R.S.*	13	45.4	115.1	23	8	0.9–1.5	1.71	68.6	+0.30
5	*S.S.*	12½	50.0	126.0	40	12½	1.9 2.8–5.0	1.68 1.68	40.0 48.0	+0.64 −0.49, +0.35
6	*W.H.*	8½	50.4	127.9	24	8½	2.5	1.50 1.50	48.0 56.0	+0.40 +0.12
7	*J.O.*	12½	60.2	152.3	34	12½	4.5	1.10	44.3	−0.13
8	*P.vB.*	13	53.4	135.4	24	8	4.3	1.42	61.7 75.7	+2.27 +1.79
9	*R.G.*	13½	44.0	111.8	16	10½	1.1	1.84	2.6	+1.20
10	*C.D.*	14½	47.5	120.6	20	10	1.9	2.45	67.0	+1.49
11	*L.B.*	11½	44.7	113.3	19	11	1.2	2.02	50.5	−0.30
12	*J.H.*	18	53.8	136.7	30	12	1.9	1.50	40.0	−0.20

confined to bed. The other two (*No. 6* and *No. 7*) were normally developed, preadolescent boys who were convalescent from rheumatic fever. At the time of the investigation their infections were entirely quiescent and their hearts were well compensated, but they were confined to bed during the studies. Although all the patients were sexually immature, there were some differences in the degree of their sexual development. *Patient No. 1* showed early adolescent enlargement of one testis. In patients *2*, *3*, *6*, *7*, the testes were of average prepubertal size. In all other cases the testes seemed abnormally small for even preadolescent boys. Five patients (*Nos. 3*, *5*, *10*, *11*, *12*) had been treated previously with methyl testosterone, and had developed small amounts of pubic hair and adolescent changes of the penis and prostate. In these cases androgenic therapy had been discontinued three months or longer prior to the present studies. The output of 17-ketosteroids before treatment is shown in Table 2.

The diagnostic classification of these patients is difficult in spite of the fact that the response to hyperglycemia (glucose tolerance curve) and response to hypoglycemia (insulin tolerance test) were studied. In no case was a gross lesion of the pituitary or brain demonstrated. On the basis of clinical observations, the following tentative diagnoses were made:

Pituitary dwarfism—*Cases 2, 3, 4, 10, 11, 12*

Delayed adolescence—*Case 1*

TABLE 2 (*Continued*)

Testosterone compounds				Androstenediol			Androstanediol		Androsterone 20 mg.i.m. 10 days	Androstenedione 20 mg.i.m. 15 days	Dehydroisoandrosterone 20 mg.i.m. 18 days
Testosterone Propionate 25 mg. (i.m.)	Methyl testosterone 25 mg. (0)	Free testosterone 25 mg. (i.m.)	Ethyl testosterone	Free 10 mg. (i.m.)	Diacetate	17-methyl	Diacetate	17-methyl			
		+++	±[3]		+[7]	±[9]					
+++[1]	+++[2]			±		±[10]					
+++	+++					+[11]					
	+++	+++				0[12]					
+++			+[4] 0[5]		+[8]	+(i.m.)[13] 0(oral)					
	+++		+[6]				+[14]	+[17]			
Not tested								0[18]			
+	±						±[15]				
	±						+[16]				
+++	+++								0		
+++	+++									0	
	+++										0

[2] Testosterone propionate, 10 mg. (i.m.) for 15 days; total, 150 mg.
[1] Methyl testosterone, 10 mg. (o.) for 20 days; total, 200 mg.
[3] Ethyl testosterone, 20 mg. (i.m.) for 7 days; 40 mg. (i.m.) for 13 days; total, 680 mg.
[4] Ethyl testosterone, 30 mg. (o.) for 15 days; 20 mg. (i.m.) plus 30 mg. (o.) for 6 days; total, 750 mg.
[5] Ethyl testosterone, 40 mg. (i.m.) for 30 days; total, 1200 mg.
[6] Ethyl testosterone, 30 mg. (i.m.) for 3 days; 40 mg. (i.m.) for 17 days; total, 770 mg.
[7] Androstenediol-diacetate, 30 mg. (i.m.) for 8 days; 45 mg. (i.m.) for 7 days; total, 575 mg.
[8] Androstenediol-diacetate, 30 mg. (i.m.) for 12 days; 45 mg. (i.m.) for 10 days; total, 910 mg.
[9] Methyl androstenediol, 30 mg. (o.) for 11 days; 50 mg. (o.) for 14 days; total 1130 mg.
[10] Methyl androstenediol, 10 mg. (i.m.) for 20 days; 40 mg. (o.) for 35 days; total, 1600 mg.
[11] Methyl androstenediol, 10 mg. (o.) for 5 days; 20 mg. (o.) for 5 days; 30 mg. (o.) for 10 days; 30 mg. (o.) plus 7.5 mg. (i.m.) for 15 days; 7.5 mg. (i.m.) for 5 days; total, 1050 mg.
[12] Methyl androstenediol, 30 mg. (o.) for 25 days; 50 mg. (o.) for 10 days; 50 mg. (o.) plus 10 mg. (i.m.) for 15 days; total, 2150 mg.
[13] Methyl androstenediol, 50 mg. (o.) for 20 days; 50 mg. (o.) plus 10 mg. (i.m.) for 5 days; total, 1300 mg.
[14] Androstanediol diacetate, 30 mg. (i.m.) for 25 days; total, 750 mg.
[15] Androstanediol diacetate, 20 mg. (i.m.) for 3 days; 40 mg. (i.m.) for 13 days; total, 580 mg.
[16] Androstanediol diacetate, 100 mg. (i.m.) for 10 days; total, 1000 mg.
[17] Methyl androstanediol, 30 mg. (o.) for 23 days; 50 mg. (o.) for 6 days; total, 990 mg.
[18] Methyl androstanediol, 40 mg. (o.) for 7 days; 50 mg. (o). for 14 days; total, 980 mg.

Normal pre-adolescent—*Cases 6, 7* (convalescent cardiac)

Froehlich's syndrome with obesity—*Case 5*

Stunted, sexually retarded, poorly nourished—*Cases 8, 9*

In the table the diet used in each test is shown calculated as the amount of protein and the number of calories per kg. of "ideal" body weight estimated for the height of the patient. In column 10 are shown the nitrogen balances which resulted from the diets during the untreated control periods prior to the various tests.

The effects of the various steroids on the retention of nitrogen are recorded as +++ indicating a marked and unquestionable increase in the retention of nitrogen, + indicating a small but suggestive effect, ± a doubtful effect or 0—no effect. The reasons for not attempting a mathematical calculation will be discussed later. The following steroids which

have been shown to have little virilizing effect were tested on one or more patients. Afterward each patient, except *No. 7* was tested similarly with a strongly virilizing androgen such as testosterone propionate, 17-methyl testosterone or unconjugated (free) testosterone.

The steroids[2] tested were:

17-ethyl Δ_4 androstene-one (3)-ol (7α) (ethyl testosterone)—*Cases 1, 5, 6*
Δ_5 androstenediol (3β, 17α)—*Case 2*
Δ_5 androstenediol (3β, 17α)—diacetate (3, 17)—*Cases 1, 5*
17-methyl Δ_5 androstenediol (3β, 17α)—*Cases 1, 2, 3, 4, 5*
Androstanediol (3α, 17α)—diacetate (3, 17)—*Cases 6, 8, 9*
17-methyl androstanediol (3α, 17α)—*Cases 6, 7*
Androstane-ol (3α)—one (17) (androsterone)—*Case 10*
Δ_5 androstene-ol (3β)—one (17) (dehydroisoandrosterone)—*Case 12*
Δ_4 androstenedione (3, 17)—*Case 11*

The dosage, method of administration and duration of treatment are shown in the footnote to the table.

RESULTS OF NITROGEN BALANCE STUDIES

Three charts are presented to illustrate the difficulty of accurately calculating the nitrogen-sparing effects of the steroids studied.

Chart 1 shows studies made on a stunted boy, aged fifteen years with delayed adolescence. It is seen that throughout the first control *Period A* there was almost a nitrogen-equilibrium and a constant body weight. In *Period B* the intramuscular administration of androstenediol-diacetate was accompanied by a light retention of nitrogen and a gain in weight. On its withdrawal in *Period C* the nitrogen balance shifted slightly to the negative side and there was small loss of weight. During the administration of methyl androstenediol in *Period D* there were fluctuations in the nitrogen balance. If a calculation were made using the balance of *Period C* as a base line, it would appear that methyl androstenediol caused a slight retention, whereas if one calculated from the original base line of *Period A* no effect would be apparent. There was a slight gain in weight during the administration of methyl androstenediol and a loss on its withdrawal. That the compound was properly absorbed is demonstrated by a marked increase in the excretion of creatine during this period. In marked contrast to the small or questionable effects of these weakly virilizing steroids was the marked retention of nitrogen and rapid gain in weight which occurred on the administration of unconjugated testosterone during *Period F*. On discontinuing testosterone in *Period G* there was a striking loss of weight

[2] These steroids were especially prepared and generously furnished by Drs. Ernst Oppenheimer and C. R. Scholz of the Ciba Pharmaceutical Products, Inc.

and a "rebound" excretion of nitrogen causing a negative balance. Unfortunately, ethyl testosterone was begun in *Period H* while the patient still had a negative nitrogen balance resulting from the withdrawal of testosterone. Because of the difficulty of selecting the proper base line for each

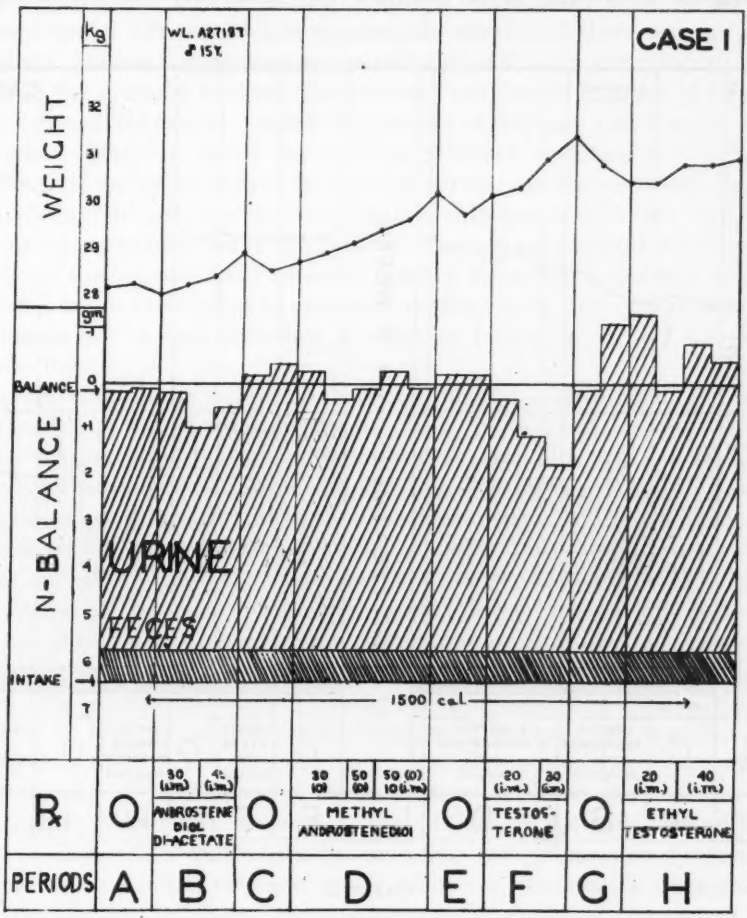

CHART 1.

period of study and because of fluctuations in the nitrogen balance during the periods of treatment, we felt that it was unwise to attempt a mathematical calculation of the effects of each steroid in terms of the amount of nitrogen retained. We prefer to evaluate the results in this

case as: androstenediol-diacetate +, methyl androstenediol ±, testosterone + + +, ethyl testosterone ±.

Chart 2 shows studies made on a sexually infantile dwarfed boy, aged fifteen and a half years, who was believed to have a pituitary deficiency. During the last 10 days of the control *Period A*, a fairly constant nitrogen

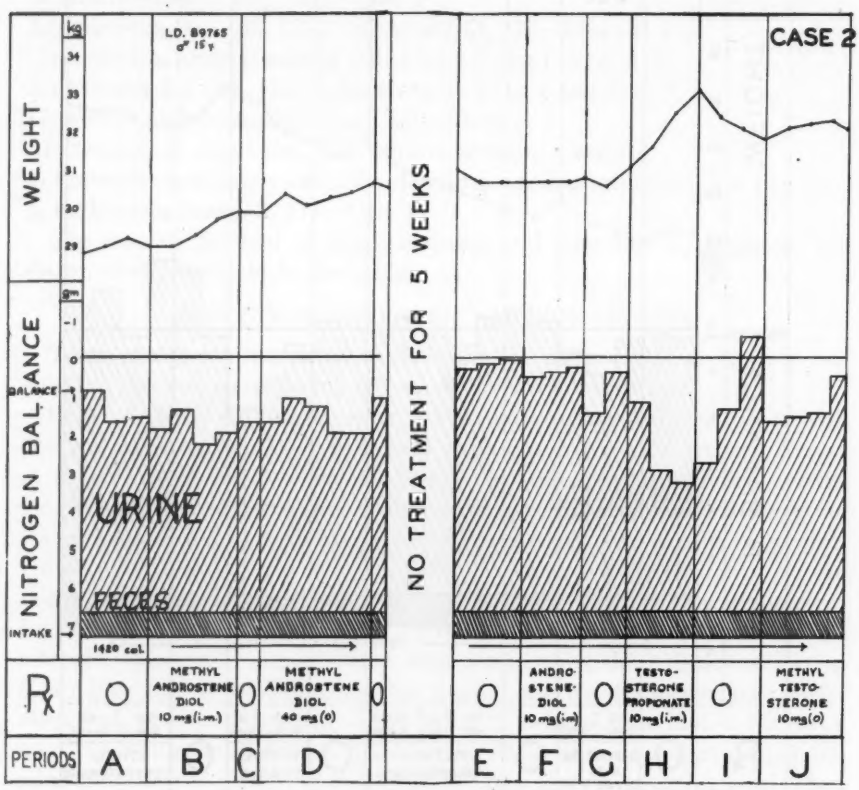

CHART 2.

retention was found which indicated a positive balance of 1.8 gm. per day. When 10 mg. of methyl androstenediol were given intramuscularly during *Period B*, and 40 mg. by mouth during *Period D*, there were irregular fluctuations in the nitrogen output making the results difficult to interpret although the general average during these periods might suggest slight

retention. There was a gradual gain in weight during these periods. Treatment was now omitted for five weeks and the patient remained at home on an unrestricted diet. On returning to the hospital, the same diet which had caused a decided retention of nitrogen during *Period A* now resulted in a nitrogen equilibrium during *Period E*. Treatment with 10 mg. of androstenediol (*Period F*) had quesionable effect and there was no gain in weight. During the subsequent control *Period G* there were unexplained shifts in the nitrogen balance. Testosterone propionate in doses of 10 mg. daily (*Period H*) caused a marked retention of nitrogen and a rapid gain in weight. *Period I* shows the rebound nitrogen excretion with loss of weight. It is unfortunate that this control period was not continued longer than 15 days in order to establish again a nitrogen equilibrium, but the patient was anxious to leave the hospital. There is no question that 10 mg. of methyl testosterone daily caused a marked retention of nitrogen during *Period J*, but a calculation to compare its effect with that of testosterone propionate would vary according to whether *Periods E, G* or *I* are used as base lines.

The results can be expressed best as: methyl androstenediol ±, androstenediol ±, testosterone propionate + + +, methyl testosterone + + +.

Case 6 (**Chart 3.**) is that of an immature boy, aged nine years, of average size who presented no endocrine abnormali y. During the control *Aeriod N* there was a small positive balance. Treatment with ethyl testosterone in doses of 40 mg. intramuscularly caused a moderate increase in retention during *Aeriod D*. During the subsequent control *Aeriod C*, the boy's hunger necessitated an increase in his caloric intake from 1200 to 1400 calories although the protein content of the diet was kept the same. This resulted in slightly less nitrogen retention in the last five days of the control period. The administration of 17-methyl androstanediol in *Period D* was followed at first by an increased retention. However, this was poorly sustained as there were increasing outputs of nitrogen during the remainder of the period. In the control *Period E* which followed there seemed to be a slight rebound with a return to equilibrium at the end. During the first five days when androstanediol-diacetate was administered (*Period F*) there was a marked nitrogen deficit which we were unable to explain. This was followed by slight retention during the rest of the treatment in which there was a gain in weight. Control *Period G* illustrates the difficulty of again establishing the same base lines as in previous control periods. Although no treatment was given for 30 days, the patient remained in a slightly negative nitrogen balance. Accordingly, it is difficult to decide whether the decrease in the nitrogen output brought about by methyl testosterone in *Period H* should be referred to *Period G* or *Period C*.

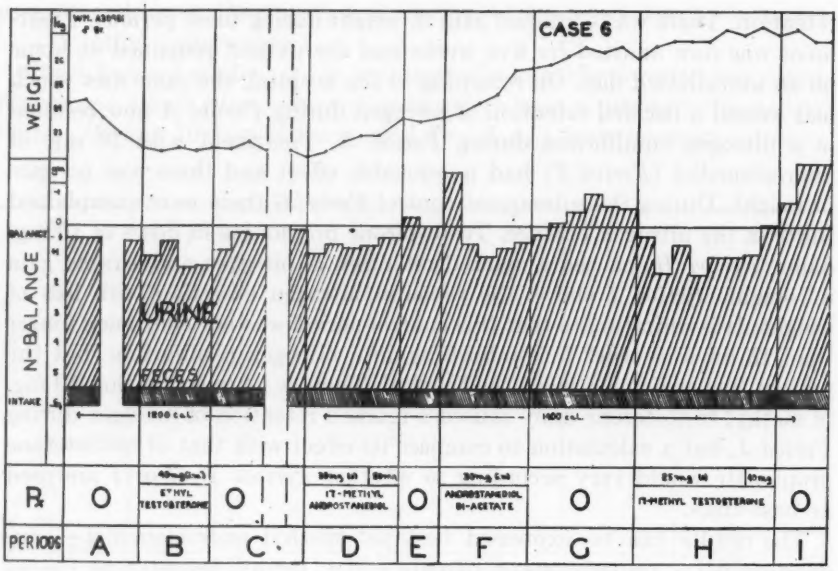

CHART 3.

The findings in this case were recorded as: 17-ethyl testosterone+, 17-methyl androstanediol+, androstanediol-diacetate+, 17 methyl testosterone+++.

Lack of space prevents the reporting of other cases in detail and the reader is referred to Table 1 for the findings.

DISCUSSION AND SUMMARY OF STUDIES ON NITROGEN BALANCE

We had hoped to evaluate quantitatively the protein anabolic effect of each steroid studied in terms of the amount of nitrogen retained per kg. of body weight. The three cases presented above illustrate some of the difficulties which would attend such a calculation. Methyl testosterone and testosterone propionate in the doses usually given cause changes in the nitrogen balance of such magnitude that they are readily apparent. However, if a steroid has only slight influence on the nitrogen balance, the effect may be obscured by other factors and the interpretation of changes may be open to question. In retrospect, it is easy for us to find criticism with our own work. It is possible that analysis of the diets for the nitrogen content instead of calculation from food tables would have yielded more exact results. It is obvious that in many instances the control periods between the administration of two different steroids should have been continued for much longer times in order to allow the nitrogen balance to

become stabilized at the same level as that found originally before treatment. In some cases (see *Case 6, Period G*) such a readjustment after discontinuing treatment is not attained in even 25–30 days. Furthermore, it is apparent that although most of our patients were quite undeveloped sexually, they did not present identical conditions. Some were possibly cases of pituitary dwarfism, others were instances of delayed adolescence, and several were normal preadolescent boys. However, even if all such errors in technic were excluded, there are a number of inherent difficulties which stand in the way of a quantitative evaluation of anabolic effects, viz.:

1. In children there are undoubtedly differences between individuals in the ability to retain and utilize protein for the purposes of growth, and there are likewise variations from time to time in the same individual. This is shown by the fact that on the same diet a patient from time to time may show quite different nitrogen balances as shown in Chart 2, *Periods A* and *E*.

2. The amount of protein stored probably is influenced to some extent by the nitrogen intake. The amount required to bring about nitrogen equilibrium might not be sufficient to permit growth. It is impossible to predict theoretically for a given individual either the minimal or the optimal protein requirements for tissue building. In fact, in children we have found it difficult to adjust a diet so as to establish nitrogen equilibrium. Therefore it is impossible to establish a standard physiological state of metabolism in each patient which would serve as a base line from which to measure the effect of an anabolic stimulant.

3. In addition to the fundamental difficulties mentioned above, many factors, often not apparent, such as minor infections, emotional changes, the amount of exercise, may cause slight fluctuations in the nitrogen output. It is rare that a constant balance can be maintained over an extended period of study. These fluctuations both with and without treatment make the exact mathematical calculation of the effects of treatment quite unreliable.

For the reasons stated, it seemed unwise to attempt to calculate from our data the exact amounts of nitrogen which were retained in each of the experiments. Nevertheless, in some tests there appeared to be an effect upon the nitrogen balance and in other tests no effect. Our observations are shown in Table 1 and may be summarized as follows:

Testosterone propionate and methyl testosterone

Either one or both of these compounds were tested on ten of the patients in the present series. In all but two cases there were marked nitrogen retention, rapid gain of weight and a "rebound" increase of nitrogen excre-

tion on discontinuing treatment. The two patients (*Nos. 8* and *9*) who showed little response were frail, poorly nourished boys. *P. v B.* (*No. 8*) had a tendency to a mild hypoplastic anemia. The B.M.R. was elevated and the serum cholesterol was low, but there were no characteristic signs of hyperthyroidism. Balance studies indicated that both boys were retaining nitrogen during the control period but that treatment caused very little increase in the amount retained. It is possible that the diets given were not adequate to permit an increased storage of nitrogen. On the other hand, one must consider the possibility that some individuals may have a fundamental disturbance of metabolism preventing the utilization and synthesis of amino acids into tissue protein.

Free testosterone

This was given intramuscularly to two patients (*Nos. 1* and *3*) in doses of 20 mg. The effects on the nitrogen retention and weight were approximately of the same magnitude as those of methyl testosterone and testosterone propionate.

17-Ethyl testosterone

This was given to three patients. A patient with retarded growth and delayed adolescence, *No. 1*, received from 20 to 40 mg. daily by the intramuscular route. A normal preadolescent boy, *No. 6*, received 40 mg. intramuscularly, and a patient with Froehlich's syndrome, *No. 5*, received 30 mg. orally and later, 20 mg. intramuscularly. In two of these cases (*5* and *6*) there appeared to be a moderate retention of nitrogen but no striking effect on the weight curve. The effect of the nitrogen retention was much less than that exerted by methyl testosterone or testosterone propionate. We know of no other studies of the effect of this compound on nitrogen metabolism.

Androsterone

This was given to one sexually infantile dwarf (*No. 10*) intramuscularly, to the amount of 160 mg. in 10 days. Although an increase in the excretion of 17-ketosteroids indicated that the compound was absorbed, there was no effect on the nitrogen balance or weight. Bassett (18) observed no effect when doses of from 12.5 to 25 mg. were given in one case of Cushing's syndrome. It is possible that larger doses or a longer period of treatment might give more favorable results but only small amounts of this steroid were available for study.

Δ_4Androstenedione

This was given intramuscularly in doses of 20 mg. for 10 days to one sexually immature dwarf (*No. 11*), with negative results. Kenyon (12a) has recently treated two women with this compound, and found small re-

tentions of 15 and 23 mg./kg. per day. The effect of the compound, however, was studied by Kochakian (15) in two castrate dogs in 1936, and he found a maximum retention of 50 and 60 mg. per kg. per day, but no effect in a normal male dog.

Δ_5Dehydroisoandrosterone

This, in doses of from 10 to 40 mg. per day was given intramuscularly to a sexually infantile dawrf (*No. 12*). He received a total of 330 mg. in 18 days. The 17-ketosteroids were increased, but there were no effects on the nitrogen retention. Negative results have been recorded by Albright (18) and by Bassett (18) each in a case of Cushing's syndrome, and by Kenyon (18) in two patients with hypogonadism.

Δ_5Androstenediol (unconjugated)

This was given in doses of 10 mg. (intramuscularly) for 15 days to one sexually immature dwarfed boy (*No. 2*). There was no appreciable effect on nitrogen retention or weight. Albright (18) treated one patient with senile osteoporosis with this compound in doses of 60 mg. per day and found no effect.

Δ_5Androstenediol diacetate

This was given to two patients (*Nos. 1* and *5*). by the intramuscular route in doses increasing from 30 mg. to 45 mg. In one case treatment was continued for 15 days, in the other 22 days. A marked increase in the 17-ketosteroid excretion was noted in both cases. In both cases there was a slight retention of nitrogen and gain in weight, followed by an increased nitrogen output and loss of weight on withdrawal.

17-Methyl $\Delta_5$androstenediol

This in five cases was given orally in doses of from 30 to 50 mg., supplemented during the last 5 to 10 days by the intramuscular injection of from 7.5 to 10 mg. The fact that it was absorbed was proved by the production of a marked creatinuria, as in the case of other androgens having a methyl group in the 17-position, while the 17-ketosteroids were not increased. One patient (*No. 3*) showed a moderate increase of the nitrogen retention and a gain of weight. In another patient, *No. 5*, there was a moderate response when the steroid was given intramuscularly in doses of 20 mg. daily. However, later when 50 mg. were given orally there was no retention of nitrogen. The failure on this occasion may have been due to the fact that treatment was begun at a time when the patient was showing a negative nitrogen balance. In two other patients, *Nos. 1* and *2*, the response to methyl androstenediol was slight and open to question. In the 5th patient, *No. 3*, no definite effect could be demonstrated.

Androstanediol and 17-methyl androstanediol

These substances have aroused considerable interest because Kochakian's (16) studies of the "renotropic-androgenic ratio" suggested that they might cause tissue building while having relatively slight androgenic effect. Since androstanediol is relatively insoluble, androstanediol diacetate which is readily soluble in oil and can be given intramuscularly in large doses, was employed. The absorption of this compound was proved by the finding of a high output of 17-ketosteroids. 17-Methyl androstanediol was given by mouth and in each case caused an increased excretion of creatine.

Androstanediol diacetate

This steroid was given to three patients. One normal preadolescent boy (*No. 6*) who received 30 mg. daily showed a small retention of nitrogen which diminished toward the end of the period and was followed by a nitrogen deficit. There was a rapid gain of weight during treatment. Another stunted, poorly nourished, sexually retarded boy, *P. v B.* (*No. 8*) when first treated with 40 mg. showed a slight and questionable retention of nitrogen and a slight gain of weight. A characteristic rebound of nitrogen excretion did not occur when the treatment was discontinued. It is of interest that this patient showed little response to testosterone propionate or methyl testosterone. The third patient, a sexually infantile, dwarfed, poorly nourished boy (*No. 9*) was treated with much larger doses, 100 mg. daily for 10 days. Even with this dose, there was only a moderate nitrogen retention but a rapid gain of weight. However, when treatment was discontinued, the retention of nitrogen continued for at least 15 days and the 17-ketosteroid excretion remained elevated, suggesting that there may have been a gradual absorption from the tissues of the large amounts of oily solution injected.

Albright (18) reported that androstanediol (unconjugated) in doses of 10.7 mg. per day (i.m.) caused a patient with Cushing's syndrome to retain daily only 5 mg. nitrogen per kg. of body weight, and Kochakian and Bassett (17) obtained a positive result in one patient. However, Williams *et. al.* (23) have recently reported a patient with Addison's disease who excreted increased amounts of nitrogen when treated with 80 mg. of androstanediol daily.

17-Methyl androstanediol

This steroid was given orally to two patients. In one normal, preadolescent boy (*No. 6*) small amounts of nitrogen were apparently retained but the retention seemed to decrease as treatment was continued. The apparent gain in weight could be accounted for by the fact that the diet had been

increased from 1200 to 1400 calories. Another normal, preadolescent boy (*No. 7*) showed no apparent response but the metabolic studies were unsatisfactory in his case.

Bassett (2) treated one eunuchoid patient with 17-methyl androstanediol and found slight nitrogen retention. Williams *et al.* (23) found no significant effect in two patients suffering from Addison's disease.

Conclusions

Our observations led us to conclude that 17-ethyl-testosterone, androstenediol diacetate, 17-methyl androstenediol, androstanediol diacetate and 17-methyl androstanediol, in some cases caused changes in the nitrogen balance similar to those brought about by testosterone propionate and methyl testosterone. The effects, however, were much smaller and less well sustained than those of the stronger androgens, and they were not uniformly accompanied by gains in weight. To give merely a general idea of the difference in magnitude, we have found that when calculated by the same methods testosterone propionate and methyl testosterone in doses of 25 mg. caused retention of from 57 to 107 mg. of nitrogen per kg. per day, with an average of 76 mg./kg./day, whereas with the weaker androgens the retention never exceeded 26 mg./kg./day. One could not be sure whether these small increases in the nitrogen balance represent true anabolic effects of the steroid, or whether they were within the range of error of the methods of measurement used.

THE EFFECTS OF PROLONGED TREATMENT WITH METHYL ANDROSTENEDIOL AND OF METHYL ANDROSTANEDIOL ON GROWTH AND WEIGHT

It seemed of importance to determine whether the androstenediol and androstenediol compounds when given over considerable periods of time were capable of causing acceleration of growth, gain in weight or other clinical effects.

Patient No. 2, whose rates of growth and gain had been followed for many years, was used as a subject. Periods of treatment with 17-methyl androstenediol and 17-methyl androstanediol were compared with untreated periods and with periods during which methyl testosterone and chorionic gonadotrophin were administered. The 17-methyl derivatives were used because they could be given orally and did not require hospitalization. The results are shown in Chart 4. Between May, 1941, and May, 1942 (*Period A*) the height increased from 49.0 to 49.7 in., and the weight from 29.3 to 29.7 kg. From June 3 to July 26, 1942 (*Period B*) the patient was treated in the hospital with methyl androstenediol orally and intramuscularly. There was slight gain of weight but no acceleration of growth. The patient was again in the hospital from September 4 to November 28,

1942 (*Period D*). During this time he received daily injections of 10 mg. androstenediol for 12 days, then testosterone propionate 10 mg. i.m. for 15 days, and finally methyl testosterone 10 mg. orally for 18 days. The periods of treatment were interrupted by control periods of ten days. The growth curve was not materially affected by these short periods of treat-

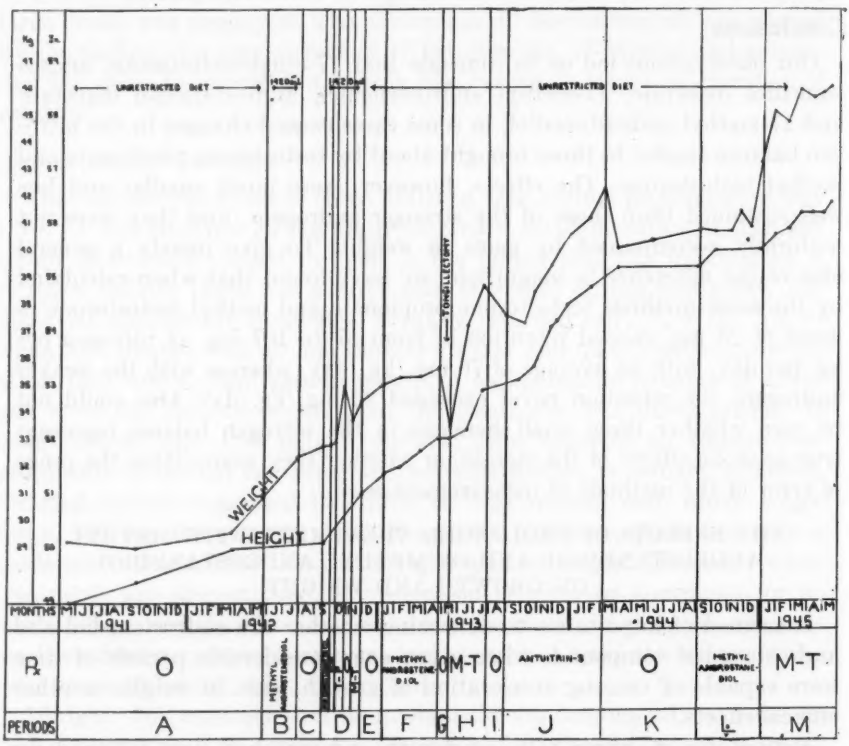

CHART 4. *Case 2.* Effects on growth and weight induced by 17-methyl androstenediol and 17-methyl androstanediol compared to methyl testosterone, testosterone propionate and chorionic hormone.

ment. During treatment with testosterone propionate there was a rapid gain in weight followed by a loss of weight when treatment was discontinued. During the two periods of study in the hospital (*Periods B* and *D*) the patient received a diet of 1420 calories. After discharge from the hospital in November, 1942, the subsequent periods of treatment were carried out at home, and the patient was allowed to have an unrestricted diet. From January 16 to April 25, 1943, a period of 14 weeks (*Period F*),

the patient was treated at home with 30 mg. daily of **methyl androstenediol** given by mouth. The rate of growth was not appreciably affected and there was no significant increase in weight. Following a tonsillectomy on April 27 the patient lost 1.5 kg. From May 25 to July 17, 1943 (*Period H*), a period of eight weeks, the patient was given methyl testosterone by mouth in doses of 25 mg. daily. There was a rapid gain of weight and a slight acceleration of growth. On discontinuing treatment there was a loss of weight and a slowing of growth. From September 4, 1943 to March 10, 1944 (*Period J*) he was given intramuscularly injections of 500 units of human chorionic gonadotrophin (Antuitrin S) three times a week. During this period there was a rapid gain of weight with a marked acceleration of his rate of growth. Pubic hair become more abundant and the genitalia increased in size. During the next six months, March to September, 1944 (*Period K*) no treatment was given; there was little change in his height or weight. For 18 weeks, from September 2, 1944, to January 6, 1945 (*Period L*), the patient received 40 mg. of **methyl androstanediol** by mouth daily. This had no apparent influence on the growth or weight curves. In contrast to this, treatment with 25 mg. methyl testosterone by mouth for another period of 18 weeks (*Period M*), from January 6 to May 12, 1945, again caused a spurt of growth and a rapid gain of weight.

In these experiments neither methyl androstenediol in daily doses of 30 mg. orally (*Period F*), nor methyl androstanediol in daily doses of 40 mg. orally (*Period L*) had any effect on the patient's growth or ability to gain weight, although methyl testosterone in doses of 25 mg. orally (*Periods H* and *M*), and chorionic gonadotrophin, 500 units 3 times a week (*Period J*) caused definite spurts of growth, gain of weight and increased muscular development. Dr. E. C. Reifenstein (19) gave 200 mg. methyl androstanediol daily to a normal adult female for one month and found no significant change in weight and no evidences of masculinization. One must conclude that in the doses given, neither methyl androstenediol nor methyl androstanediol had significant therapeutic value as stimulants of protein anabolism. The small increments of nitrogen retention which were apparently demonstrated in some of our patients by the method of measuring changes in the nitrogen balance while a constant diet was given, might have been due to errors in our methods of study. However, it seems more probable that there were actually retained small increments of nitrogen in the early stages of treatment, but that these effects were not sustained for any length of time, and in the long run, were insufficient to cause any clinical effects in the patient. The tendency for the nitrogen retention to decrease when treatment with the androstanediol compounds is continued, is shown in *Case 6, Periods D* and *F*.

SUMMARY

The influence on protein anabolism of nine different weakly androgenic steroids of the androstene or androstane series was studied in immature boys. The steroids, 17-ethyl testosterone, Δ_5 androstenediol (3β, 17α) diacetate (3, 17), 17-methyl Δ_5 androstenediol (3α, 17α), androstanediol—(3α, 17α)—diacetate (3, 17) and 17-methyl androstanediol—(3α, 17α), in some cases caused changes in the nitrogen balance similar to those brought about by testosterone propionate and methyl testosterone. The effects, however, were much smaller and less well-sustained than those of the latter and they were not uniformly accompanied by gains in weight. In one patient, 17-methyl androstenediol and 17-methyl androstanediol were given over considerable periods of time. In doses which it would be practical to give, neither compound seemed to be of therapeutic value as a stimulant of protein anabolism and of growth.

ACKNOWLEDGMENTS

The methyl testosterone (Metandren), the testosterone propionate (Perandren) and the weaker androgens used were kindly supplied by the Ciba Pharmaceutical Products, Inc., Summit, New Jersey. We wish to express our appreciation to Drs. E. Oppenheimer and C. R. Scholz of this company for their valuable advice and interest in this work. We wish to thank Drs. Fuller Albright, A. T. Kenyon and E. C. Reifenstein, Jr., for reading and criticizing our manuscript.

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PROGESTERONE THERAPY IN UTERINE FIBROMYOMA

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IN A STUDY on experimental fibromyomas and their prevention, Lipschütz and Inglesias (6) showed that subserous uterine fibromyomas and extragenital subserous fibromyomas in the abdominal cavity can be produced in guinea pigs subjected to prolonged treatment with estrogens. All estrogens, natural and artificial, free and esterified, were shown to be tumorigenic. Under certain conditions of treatment with estrogens a female guinea pig only rarely escaped the development of abdominal fibromyomas. Continuous action of the hormone was an essential factor. When a period of one week of injections was followed by a period of two weeks without injections no fibromyomas were produced even after a year of such treatment. On the other hand, minute quantities of free estradiol, insufficient to produce fibromyoma when given three times weekly by subcutaneous injection, proved highly active when absorbed from a subcutaneously implanted pellet. It was suggested that the tumorigenic action of estrogens in the normal organism is controlled by the sexual cycle with the alternation of high and low level phases of blood estrogen and by inactivation or elimination of the hormone.

Uterine and extra-uterine abdominal fibromyomas produced in the guinea pig by subcutaneous injection of follicular hormone develop in the non-castrated female with less constancy and in lesser degree than in the castrated female. This suggests that the luteal hormone may be responsible for the antitumorigenic influence of the ovary. This was corroborated in a later study by Lipschütz, *et al.*, (9) who demonstrated that progesterone[1] prevented tumor formation by estrogens.

They hypothesize (5) that the development of uterine fibromyomas in women is due to disturbance of the normal balance between follicular and luteal hormones and of their normal timing, and that progesterone may prove useful as a therapeutic agent against fibromyoma.

Postulating that progesterone produced in the body may react synergistically with estrogen on the uterine mucosa but also have an antagonistic action, Lipschütz and his coworkers (10) investigated the preventive action of several steroids, including progesterone, on such experimentally produced tumors. Three different synthetic steroids—progesterone, desoxy-

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[1] Acknowledgement is made to Ciba Pharmaceutical Products, Inc. for the generous supply of progesterone (*Lutocylin*).

corticosterone acetate, and testosterone propionate were shown to have the capacity of preventing abdominal fibroids elicited by estrogens. The antifibromatogenic threshold, or the minimal quantity of the steroid which must be released to inhibit fibroids, was lowest with progesterone; desoxycorticosterone acetate was next in order, and was followed by testosterone propionate which was still highly active. The question was raised whether the descending order of the antifibromatogenic faculty of different steroids was coincident with their anti-estrogenic activity. This was the case in the guinea pig but not in the rat. The progestational activity of the three compounds appears to decrease in the same order as the antitumorogenic effect although these quantitative data are by no means exact. It seems somewhat contradictory, according to Lipschütz (5), that the progestational and antifibromatogenic activities should be of the same magnitude, since progestational activity is associated with stimulation of the proliferation of epithelial cells, whereas antifibromatogenic activity indicates inhibition of the proliferation of those cells which give origin to fibroblasts and connective tissue fibers. However, it has been suggested that both stimulation and inhibition of proliferation may depend upon different quantitative levels of the antagonistic substances in play. These results are in accord with clinical observations that uterine fibromyoma are most frequent in virgin women, and that these tumors are more often present in nulliparas than in women who have given birth to children. Their frequency is also reported to be greater in women who became pregnant only at a more advanced age and had fewer pregnancies. It is assumed that the longer action of progesterone may exert a preventative influence in women with a greater number of pregnancies.

As experimental fibroids elicited by estrogens are structurally different from uterine fibroids in women, Lipschütz questions (5) whether the above findings can be applied to the treatment of fibromyomata in women. But attention is called to the fact that the fibromyoma in women, like the experimental fibroids of the guinea pig, regresses when production of estrogen is suspended or diminished, as after the menopause, and after castration or ovarian irradiation. As progesterone is the most potent antifibromatogenic steroid, he suggests its therapeutic use.

Realizing that the application of laboratory results to the human female should be made with caution, the author was stimulated by Lipschütz's extremely careful investigations to extend the studies into the clinical field with special reference to the therapeutic possibilities of progesterone.

It would have been highly desirable to have objective measurements of the tumors by making tracings of the uterine contour, injecting radiopaque material into the uterus, sounding the uterus, etc., but such methods were difficult to employ with the facilities that were available.

All individuals in the series were charity patients of the clinic, and great difficulties were encountered in obtaining accurate data and regular attendance to the clinic. A clinical trial with progesterone in the following seven patients with uterine fibromyomata was undertaken:

CASE HISTORIES

Case 1

E. W., a white female, aged 31, was first seen on November 11, 1944, complaining of an increasing vaginal discharge of several months' duration. There was nothing unusual in her menstrual history. She started to menstruate at 13, periods occurred approximately every 28 days, lasting five days. No pregnancies.

Physical examination revealed nothing abnormal except in the pelvic examination. The introitus was patent and the perineum firm. When the cervix was exposed with a speculum, the lips appeared to be turned apart by what seemed to be a cervical fibromyoma approximately 3 cm. in diameter, extending through the external os and attached to the dorsal surface of the uterine canal which was otherwise normal.

Diagnosis: Fibromyoma of the uterus.

Treatment: On November 13, 1944, injections of progesterone in doses of 10 mg. three times weekly were begun. After three injections a definite change could be observed in the tumor. There was shrinkage, and slightly gray trabeculae were seen over the outer surface. The tumor gradually diminished in size, and by January 5, 1945, it had shrunk to about one fourth of the original size, allowing the cervical lips to approximate. Progesterone was discontinued for one month and then resumed. On March 1*st* there was seen a slight elevation of the lining of the canal with complete closure of the cervical lips. The original site of the tumor was firm, with gray trabeculae of fibrous tissue formation. The vaginal discharge ceased, and there was no change in the regularity of the menstrual cycle.

Case 2

W. W., a white female, aged 47, was first seen on January 30, 1945, complaining of heaviness of the lower abdomen, "bearing-down" sensation, pressure on the rectum, and an increase in frequency of urination of a year's duration. Except for the above, the past history was irrelevant. Menarche started at the age of 16 with regular periods every 28 days. She had one child at the age of 26; no miscarriages. About eight months previous to the first visit her periods started to lengthen, and for the past six months she had flowed constantly in varying amounts. With the change in periods there occurred hot flashes, a tight sensation at the base of the head and neck, a tingling of extremities and increased nervousness.

Physical examination revealed a short, obese, apprehensive female. The head, neck, heart, and lungs were normal. The abdomen was obese and pendulous. There was a large mass palpable over the pubis, halfway to the umbilicus, extending into both lower quadrants. The perineum was moderately relaxed, the cervix was enlarged and it pressed down into vagina but was not fixed. There was a slight endocervicitis. The uterus was enlarged, approximating the size of a three-months' pregnancy; it was movable, smooth, and very hard. There was tenderness along ligaments. The ovaries and tubes appeared to be normal. Moderate varicosities were present on both lower extremities.

Diagnosis: Uterine fibromyoma; slight endocervicitis; menopause.

Treatment was started on January 30, 1945 with injections of progesterone in doses

of 10 mg. six times weekly. By February 13*th* the tumor had decreased about 40 per cent in size; menstrual bleeding had ceased. On February 27*th* there occurred a normal period without discomfort. The patient's nervousness and hot flashes were markedly relieved. The uterus was no longer palpable by abdominal examination, and on vaginal examination it appeared to be the size of a lemon.

Case 3

F. W., a colored female, aged 24, was first seen on July 25, 1944, complaining of intermittent attacks of cramplike, "bearing-down" pains across the lower abdomen and lumbar area of two weeks' duration. Menses had been irregular for the previous two months, with a profuse flow occurring daily with many large clots and usually lasting two weeks. One week prior to her visit a profuse, yellowish vaginal discharge was noted. The patient had had three pregnancies, the first occurring at the age of 14 years.

Physical examination was normal except for the pelvic examination which revealed moderate tenderness suprapubically. The cervix was indurated, indented and slightly enlarged. The uterus was hard, immobile, and was enlarged to the size of a large grapefruit.

Diagnosis: Fibromyoma of the uterus, degenerative type; cystocele.

Treatment: Patient was considered inoperable because of the fact that the uterus was bound down, firm, and immobile. Injections of progesterone, 10 mg. daily, were started on July 28, 1944. On August 2*nd*, she was improved, and was discharged from the hospital; there had been a decrease in the size of the uterus. On August 15*th* the uterine mass was considerably smaller, and the right adnexa were palpable. This was impossible at the start of the treatment. On August 22*nd* the uterine fibroid had decreased in size about 25 per cent. The patient failed to return for further treatment.

Case 4

O. J., a 24 year old colored female, was first seen in August, 1943, complaining of cramps with her menstrual periods. Examination showed the uterus in good position and of normal size. In April, 1944, she bled for two weeks and had cramplike pains. Since July, 1944, she experienced upper abdominal pains, and on October 1*st* there occurred a normal menstrual period, but the abdominal pains persisted. Because of this she again sought medical advice on October 17*th*.

Physical examination revealed a small, deep suprapubic mass. The introitus was patent and the perineum relaxed. The cervix was slightly enlarged and had a mild endocervicitis. The uterus was hard and smooth, and was enlarged to the size of a two-months' pregnancy.

Diagnosis: Uterine fibromyoma; endocervicitis.

Treatment: Progesterone in doses of 10 mg. three times a week, was started on October 17, 1944. The patient was not seen again until December 5*th*, being on constant treatment. She reported two normal periods with flow lasting three days, and there was no distress of any kind during this time. The tumor mass had decreased by 40 per cent. Four weeks later the tumor was still of the same size, after which the patient failed to return for further treatment.

Case 5

L. L., a colored female, aged 52, was first seen on August 8, 1944, complaining of lower abdominal pain with pressure on the rectum and bladder, urinary frequency with nocturia. Her past history was negative until about two years ago when pains occurred in the lower abdomen with pressure on the rectum and bladder. Her menstrual history was normal until about one and a half years ago when she began to flow excessively dur-

ing her periods, only three occurring during the past year. For the past eight to ten months, she noticed urinary frequency with nocturia. Her first pregnancy was at the age of 14 years.

Physical examination showed a large mass extending four inches above the pubis and into both lower quadrants. The perineum was moderately relaxed; the cervix was enlarged, everted and infected. The uterus contained an irregular fibromyoma which filled two thirds of the pelvis.

Diagnosis: Large uterine fibromyoma; endocervicitis.

Treatment: The patient was given injections of progesterone, 10 mg. daily, and one week later the mass appeared to be undergoing involution. By September 12*th*, the fibroid had decreased one third in size. The patient failed to return to the clinic for further treatment.

Case 6

B. C., a 34 year old colored female was seen on September 12, 1944, complaining of lower abdominal pain of five years' duration. Menarche started at the age of 12, with regular periods every 28 to 30 days, lasting three days. Moderate cramps and backache occurred with each period.

Physical examination showed a small mass palpable just over the pubis, and there was tenderness on pressure over both lower quadrants. The pelvic examination revealed a slightly relaxed perineum and slight ectropion of the cervix with endocervicitis. The uterus was enlarged to at least twice normal size; it was movable, hard and smooth. Marked tenderness was present on pressure over the ligaments.

Diagnosis: Small uterine fibromyoma; endocervicitis.

Treatment: On September 12, 1944, injections of progesterone in doses of 10 mg. three times a week were started. On September 26*th*, the uterus had decreased 30 per cent in size. On October 25*th*, after the patient had received eighteen injections of progesterone (180 mg.), examination revealed a uterus only slightly larger than normal. The patient failed to return for further treatment.

Case 7

B. E., a 26 year old colored female, presented herself on June 30, 1944, with a history of metrorrhagia. In March, 1942, she flowed excessively for three weeks, passing large clots. In August of the same year she had another similar experience. These recurred, and in July, 1943, a curettage was done. The following six periods were fairly normal, but in January, 1944, the excessive bleeding returned, and persisted. Her last menstrual period was said to have started June 16*th*, and stopped June 24*th*, only to start again the next day and persist to the date of her first visit. The patient had had a bilateral salpingectomy, and was being treated for syphilis. There was one miscarriage at the age of 14.

Physical examination showed a well healed midline scar, no abdominal masses, but there was tenderness on deep pressure both in the suprapubic region and to the right of the midline. Pelvic examination revealed a mass about the size of a small egg on the right upper half of the uterus, and there was tenderness on pressure on the ligaments.

Diagnosis: Small uterine fibromyoma.

Treatment: Injections of progesterone, in doses of 10 mg. three times a week, were started immediately, and on July 18*th*, after seven injections, the mass had decreased to about one third in size. Bleeding had stopped shortly after treatment was started, and a normal period occurred on July 14*th*. On August 1*st* the mass appeared to be com-

pletely gone; the uterus was small and slightly retroverted. The patient had received a total of twelve injections of progesterone, each 10 mg.

DISCUSSION

The reported cases have, in common, a degree of subsidence of the size of the uterus and a regulation of menstrual disorders in a period of time which, according to the author's experience, is not conformable with the assumption of a spontaneous regression of the tumor. Whether we dealt with a true regression of fibrous tissue, as demonstrated in guinea pigs by Lipschütz and his coworkers, is also not revealed by these clinical observations. One has to consider a decrease in size of the uterus or a shrinkage by dehydration with the real tumor remaining essentially intact. Changes in size of the uterus, its vascularization and its water content under the influence of steroid hormones are so well established that one has to consider the possibility that simple clinical examination under not sufficiently controlled conditions may lead to misconceptions.

From such a small series of cases no valid conclusions of any kind should be drawn. No efforts have been made to support the diagnosis of uterine fibromyomata by histological evidence, although in all patients there was, clinically no doubt of the presence of a seemingly benign tumor. Dealing mainly with charity patients who would not return to the hospital after relief of symptoms made continued observation for a reasonable period of time impossible; thus, it should be emphasized that with this presentation, no claim for a final therapeutic effect of progesterone in fibromyomata is raised.

Progesterone is not offered as a substitute for the presently accepted methods of surgical or radiation therapy of fibromyoma. We believe, however, that the subject and the questions raised by our findings are of sufficient interest and practical significance to be studied by investigators whose facilities permit a careful investigation with all necessary laboratory support.

SUMMARY

On the basis of the work of Lipschütz and his coworkers, who reported that fibroids, produced in the guinea pig by continued estrogen administration, decrease and disappear under progesterone (and other steroid) therapy, seven patients having fibromata uteri, clinically diagnosed, were treated by injections of progesterone. An observed decrease in the size of the tumor or of the uterus in all cases was reported with the aim to encourage further investigation of the possible therapeutic value of this treatment in such conditions.

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Abstracts of
CURRENT ENDOCRINE LITERATURE

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ENDOCRINE GENERAL

GUSBERG, S. B. Androgenic therapy of menopausal symptoms in cancer patients. *Am. J. Obst. & Gynec. 50* (5): 502–509 (1945).

The author treated 24 patients who had been subjected to surgery, radiation, or both, for neoplastic disease or functional uterine bleeding. Methyl testosterone, 20 milligrams, orally was given for four weeks and then 10 milligrams for two to four weeks. Several intervals of one week of no therapy were allowed for during the course of treatment. Twenty-three of the patients had satisfactory relief of their vasomotor symptoms. For the small number of menopausal women who require endocrine therapy, the author recommended androgens rather than estrogens in the following:

Those who have received treatment for gynecological or mammary cancer; those who have been treated for abnormal uterine bleeding during the climacteric; those who have had diethylstilbestrol-induced bleeding; those who require treatment for vasomotor symptoms before their menses have ceased; and those who have become "addicted" to diethylstilbestrol.—*C.D.D.*

HAMBLEN, E. C., AND C. D. DAVIS. Treatment of hypoovarianism by the sequential and cyclic administration of equine and chorionic gonadotropins—so-called one-two cyclic gonadotropic therapy: summary of five years' results. *Am. J. Obst. & Gynec. 50* (2): 137–146 (1945).

A total of 116 hypo-ovarian patients, whose ages placed them in the adolescent or reproductive epoch, were treated by the sequential and cyclic administration of equine and chorionic gonadotropins. The following results were obtained:

Only one of seven patients with deficient sexual maturation and non-occurrence of menarche (hypo-estrogenism) yielded a progestational endometrium during therapy. Four of thirteen patients, or 30.8 per cent, with infrequent and/or scanty estrogenic uterine bleeding yielded progestational endometriums during therapy. Fifteen of thirty-one patients, or 48.5 per cent, with prolonged and/or excessive estrogenic bleeding yielded progestational endometriums during therapy. Seven of fourteen patients, or 50 per cent, with cyclic estrogenic bleeding and ovarian sterility yielded positive responses,

including four pregnancies, during therapy. Nine of fifty patients, or 18 per cent, with cyclic bleeding from immature progestational endometriums became pregnant during therapy.

The following conclusions appear warranted: 1. Patients with hypo-estrogenism (as illustrated by those with deficient sexual maturation) respond poorly to this system of gonadotropic therapy; 2. Patients with anovulatory ovarian failure without hypo-estrogenism (as illustrated by those with diverse types of estrogenic bleeding) respond well to this system of therapy: a total of 44.8 per cent yielded progestational endometriums; 3. A small percentage (18 per cent) of sterile women, whose bleeding from immature progestational endometriums constitutes the only significant finding upon their surveys or those of their husbands, become pregnant when treated with one-two cyclic gonadotropic therapy.—*C.D.D.*

INGRAM, C. H. Therapy in habitual abortion. *Am. J. Obst. & Gynec. 50* (2): 154–159 (1945).

Six patients who had had a total of 16 pregnancies with but two living babies were treated by the following regime: Three capsules (each capsule containing the equivalent of 30 milligrams of alpha tocopherol) of mixed Tocopherols daily as soon as the first period was missed, six capsules daily for three days before the next period was due, nine capsules daily during the days of the expected flow, tapering off with six capsules for three days and then returning to the three capsule level until time for the next period; also oral pregneninolone was given 15 milligrams on the first three days of each expected period. This regime was maintained until quickening occurred. All other routine anti-abortional measures were instituted at once. All six pregnancies came to term and living babies resulted.—*C.D.D.*

LEONARDS, J. R. AND A. H. FREE. A note on gastric retention in one-hour, two-dose glucose tolerance tests. *J. Lab. & Clin. Med. 30:* 1070 (1945).

The Rose-Exton method of performing glucose tolerance tests was utilized in sixteen normal male medical students. At the end of the 60 minute period from 38 to 62 gm. of glucose were recovered from the stomach unchanged, with an average figure of 50 gm. However, the amount of glucose remaining in the stomach could not be correlated with the changes in blood sugar in the various subjects. These findings are consistent with the authors' previous observations that the average blood sugar pattern is quite similar during the first hour, irrespective of whether one or two doses of glucose are given.—*T.H.McG.*

NIEBURGS, H. E. Body temperature—a diagnostic aid in menstrual disorders and sterility. *J. Obst. & Gynaec. Brit. Emp. 52* (5): 435–462 (1945).

The body temperature was studied in 41 women in relationship to menstrual function. The actual number in each category, e.g. normal, menopausal, etc., was not given. Temperatures were taken in the evening. Observations were made during twenty-three cycles in an unstated number of normally menstruating women. Vaginal recordings were taken in many instances and were about 0.6 degrees F. less than but paralleled oral values. There was a fall in temperature for one to two days before menses, a further decrease

during the menstrual phase to even 97 degrees F., followed by a rise of one or two degrees. During the follicular phase, the first eight or ten days postmenstrually, the temperatures fluctuated between 98 and 99 degrees F. There was a sharp fall about the 14th day at the ovulatory phase and thereafter a rise during the luteal phase, reaching 100 degrees or more a day or two before the menses. In cases of secondary amenorrhoea due to protracted follicular activity the temperature was persistently low. In cases of dysmenorrhoea 38 cycles were studied and all failed to show the fall just before the menses. Administration of oestrogens always lowered the temperature, progesterone raised it. Other effects are described. The results are illustrated by 29 graphs. The author states that in amenorrhoea, oligomenorrhoea, menorrhagia and dysmenorrhoea and intermittent bleeding, the temperature curves are of diagnostic value and aid in deciding which hormone to give and the dosage.—*R.A.C.*

PARKES, A. S. Preservation of spermatozoa at low temperatures. *Brit. M. J. 2:* 212 (1945)

Human semen was frozen at temperatures of −20°, −79° and −190° C. in thin films, in small capillaries of 0.15 mm. diameter, in capillary tubes of 1 mm. diameter, and in 1 cc. ampules. In the thin films and small capillaries no active spermatozoa were obtained on thawing at 37° C. A large proportion of spermatozoa survived for long periods in semen which was frozen in bulk at either −190° C. or −79° C. The rate of freezing and thawing did not seem to be the primary factor in the survival of human spermatozoa exposed to low temperatures. The ability of spermatozoa treated in this manner to bring about fertilization of ova was not determined.—*L.T.S.*

PINCUS, G. AND H. HOAGLUND. Effects on industrial production of the administration of Δ5 pregnenolone to factory workers, I. *Psychosom. Med. 7:* 342–347 (1945).

A study of eight skilled leather cutters was made over a period of eight weeks. The men received no incentive pay and the amount of production *per se* was therefore not a measure of the effects of experimental procedures. The wastage of leather was measured and compared when the men were taking 45 mg. per day of Δ5 pregnenolone and when they were taking placebos indistinguishable from the pregnenolone pills. Pregnenolone administration was not found to lessen the wastage in cutting sole leather. Twelve turret lathe operators (eight men and four women) were studied in relation to their daily production of bayonets on a piece work incentive basis when taking pregnenolone and when they were taking placebos. During placebo administration the efficiency of production increased 10.1 per cent ±2.18 per cent. During pregnenolone administration the increase was 18.3 per cent ±1.71 per cent. The difference of 8.2 per cent is three times the standard deviation and the value of P is 0.005. The difference is therefore statistically significant. Scrapped bayonets were less by 32.7 per cent when the workers were taking pregnenolone in contrast to when they were taking placebos, but this difference between scrap figures is not quite statistically significant (P =0.06). As medication was switched the production declined in the placebo group, rose in the pregnenolone group. This switchover effect occurred for each of two medication switches made during the series of tests. On discontinuance of all medication an efficiency increase of about ten per cent remained during the four weeks the production was followed. The role of job stress and incentive is discussed in relation to the findings.—*Authors' Summary.*

PINCUS, G., H. HOAGLAND, C. H. WILSON, AND N. J. FAY. Effects on industrial production by the adminstration of Δ5 pregnenolone to factory workers, II. *Psychosom. Med. 7:* 347 (1945).

A study of piece work production has been made of 173 male and female workers making optical goods when they were taking placebos and when they were taking pregnenolone. A significant increase of 4.2 per cent in overall production was found for 77 workers taking 50 mg. pregnenolone per day over their previous production levels. This is 1.3 per cent higher than their levels on placebos. Decrease in wastage and repairs for 39 workers amounted to 18.9 per cent when they were taking pregnenolone and 13.7 per cent when they were taking placebos as compared to their pre-experimental levels. Ninety-six workers were studied for three months. A third of them took 70 mg. per day pregnenolone pills, another third took 25 mg. per day and a third took 0.1 mg. per day (a placebo for all practical purposes). The 70 mg. group showed most pronounced improvement in earnings and in the saving of wastage. The 25 mg. per day group fell between the 70 mg. per day and the 0.1 mg. per day groups. There was thus demonstrated differences in dosage on improvement of industrial production resulting from the ingestion of pregnenolone. Some improvement of production due to pregnenolone tends to be retained after the termination of medication.—*Authors' Summary.*

SALA, S. L., D. ORELLANA, AND JOSÉ M. LAZCANO GONZALEZ. An accelerated Ascheim-Zondek reaction. *Semana méd. 52* (II): 328 (1945).

Two injections of morning urine, ¾ cc. each, were made intraperitoneally in female rats 21 to 25 days of age. Inspection of the ovaries after two hours showed an increase in ovarian weight between 50 and 100 per cent and vascular congestion with known positive urine. Positive readings were obtained in 70 out of 71 cases of pregnancy, all controls of 21 non-pregnant women and seven men being negative. Agreement with the regular A–Z test was 28 in 30 cases.—*A.E.M.*

SALABER, J. A., G. DI PAOLA, J. L. AHUMADA, AND H. L. GUIXA. Treatment of disorders of the female menstrual cycle with serum gonadotrophin. *Rev. Asoc. méd. argent. 59:* 1302 (1945).

The authors have administered serum gonadotrophin to 40 women with disorders of the menstrual cycle with the following results: no effect in three cases of primary amenorrhea; immediate benefit in 33.3 per cent, and delayed benefit in another 33.3 per cent of 13 cases with secondary amenorrhea; immediate benefit in 57.1 per cent, and delayed benefit in 14.2 per cent of seven cases with metropathia hemorrhagica; immediate benefit in 57.1 per cent, and delayed benefit in none of seven cases of secondary ovarian insufficiency; and a favorable response in ten per cent of ten cases with sterility. The authors believe that serum gonadotrophin is a valuable therapeutic tool in the management of some disorders of the menstrual cycle. Those patients that show in immediate response are benefitted to a greater extent than those that show a delayed response. The mechanism through which hemorrhage is stopped in metropathia hemorrhagica is not clear since the endometrium usually does not become progestational after the therapy. The authors suggest that in women with menstrual disorders, serum gonadotrophin is relatively ineffective therapy because most of these women have disturbances which are due to conditions primary in the ovary; they believe that in the smaller group of patients

with disorders of pituitary etiology, serum gonadotrophin has a better chance of inducing a favorable response.—*J.R.R.-M.*

TRITSCH, J. E. AND E. SCHNEIDER. Clinical experience with a new synthetic ergonovine-like substance. *Am. J. Obst. & Gynec. 50* (4): 434–438 (1945).

The authors used methergine intramuscularly in 100 patients and intravenously in 100 patients. In the first group, 0.2 milligrams was given immediately following the delivery of the placenta. In the latter group, the same dosage was administered immediately following the birth of the child and before the placenta was delivered. The average blood loss was 113 cubic centimeters with the intravenous technique and 168 cubic centimeters with the intramuscular route. Manual removal of the placenta was necessary three times in the intravenous group but not at all in the intramuscular group. The authors believe that methergine is a nontoxic and efficient oxytocic drug.—*C.D.D.*

VAUX, N. W. AND A. E. RAKOFF. Estrogen-progesterone therapy: a new approach in the treatment of habitual abortion: studies indicating its rationale in 24 treated cases. *Am. J. Obst. & Gynec. 50* (4): 353–366 (1945).

Twenty-four patients, all of whom had had two or more previous abortions, were treated by the intramuscular administration of 10 milligrams of progesterone and 10,000 rat units of alpha-estradrol benzoate, two to three times weekly to the period of viability. There was a fetal salvage of 67 per cent. In 13 of the 19 patients studied, both estrogen and pregnanediol urinary excretion levels were diminished prior to therapy. Ninety-five per cent of the patients showed normal serum gonadotropins.—*C.D.D.*

VICTOR, J. Hypertension produced in dogs by unilateral ligation of peri-adrenal blood vessels and tissue. *Proc. Soc. Exper. Biol. & Med. 60* (3): 332–334 (1945).

The peri-adrenal blood vessels and surrounding tissue of the left adrenal glands of five dogs were ligated, and in one dog one artery running to the lower pole of the gland and the hilar vein were ligated. Hypertension developed within one to three days and persisted at the time of presentation of the results (two to four months following operation). Systolic pressure exceeded 100 mm. Hg. and diastolic pressures of as high as 280 mm. Hg. were observed. No significant changes in urine analysis of blood NPN levels of the hypertensive dogs were observed.—*F.N.A.*

WALKER, K. M. The diagnosis and treatment of male infertility. *Proc. Roy. Soc. Med. 38:* 243 (1945).

The author stresses the importance of semen examination, postcoital examination of cervical mucus for penetration of sperm, and testicular biopsy, under pentothal anesthesia. For treatment he recommends elimination of all forms of chronic infection, and suggests that the sulfonamides may have a temporarily adverse effect on the germinal epithelium. For staphylococcus infections as well as gram-negative organisms he suggests and prefers the use of the acetone- and alcoholic-soluble fractions of extracts of garlic. Hormone treatment is recommended for its effect on the epididymis where sperm are not

only stored, but brought to maturity. Crystals of methyl testosterone are preferred since they provide a deposit in the gluteus for a week. Androgens are indicated for 1) hypoplasia or inadequate functioning of the ducts; 2) low invasive capacity of the sperm and low viability; 3) certain cases of lowered potency and of failure to ejaculate; 4) some types of deficient spermatogenesis associated with sloughing of seminal epithelium. Dosage of methyl testosterone suggested was 1.5 to 3 mg. one to three times a week.—*R.C.*

WAY, S. Relation between gastric acidity and the anterior-pituitary-like hormone content of urine in pregnant women. *Brit. M. J. 2:* 182–184 (1945).

In this investigation the secretion of free and total acid in response to a test meal was correlated with the secretion of APL hormone in the urine of women in different stages of pregnancy. An inverse correlation was found between the concentration of anterior-pituitary-like hormone and the free and total hydrochloric acid. It is thought that the hypochlorhydria of pregnancy is connected with this correlation.—*L.T.S.*

WINOCUR, P. Growth in obese children. Study of the height of 4,629 obese children. *Prensa méd. argent. 32:* 2322 (1945).

The author has analyzed the weight and height of 34,172 Argentine children between the ages of three and 16 years who were residents of the city of Buenos Aires. In this group there were 4,169 (13.5 per cent) whose weight exceeded the average normal in relation to their chronological age by 20 to 37 per cent. The height of these obese children exceeded the normal in all of the age groups studied. The author stresses the correlation between weight and height in these children: those who were overweight were also overheight. These children are being observed to determine whether the increase in height is due to growth that begins early and ceases prematurely, or to growth that results in a final stature that exceeds the height of the average adult. A correlation will also be made between the final height achieved by these children and that anticipated from their hereditary antecedents.—*F.A.de laB.*